INFLUENCE OF NUTRIENT DENSITY AND FEED FORM ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND GASTRO INTESTINAL TRACT DEVELOPMENT IN BROILERS FED WHEAT-BASED DIETS

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science
in
Animal Science

at Massey University, Manawatu
New Zealand

Obright Hamungalu

2019

Abstract

This study investigated the influence of nutrient density and feed form, and their interaction on growth performance, nitrogen-corrected apparent metabolisable energy (AMEn) and upper gut components of broilers fed wheat-based diets for 35 d post-hatch. A completely randomised design was used, with a 5 x 2 factorial arrangement of 10 treatments (with 6 replicates, 8 birds per replicate) involving five dietary nutrient density, VL, very low nutrient density (11.71 MJ/kg AMEn, 13.44 g/kg lysine); L, low nutrient density (12.13 MJ/kg AMEn, 13.92 g/kg lysine); M, medium nutrient density (12.55 MJ/kg AMEn, 14.40 g/kg lysine); H, high nutrient density (12.97 MJ/kg AMEn, 14.88 g/kg lysine); VH, very high nutrient density (13.39 MJ/kg AMEn, 15.36 g/kg lysine) and two feed forms, mash *vs.* pellet. Nutrient density x feed form interaction was significant ($P < 0.05$) for weight gain and feed intake during finisher and whole grow-out period, while during starter period the interactive effect was significant ($P < 0.05$) for weight gain. At each nutrient density level, weight gain and feed intake were higher in birds fed pelleted diets than those fed mash diets, but the advantages of pelleting were greater at the lowest nutrient density. During whole trial period feed per unit gain (F/G) significantly ($P < 0.001$) improved as the nutrient density level increased, but it deteriorated ($P < 0.05$) due to pelleting. Effect of feed form was significant ($P < 0.01$) on bird uniformity, with pelleting having 10 % higher uniformity compared to mash diets. Increasing nutrient density had a significant ($P < 0.001$) effect on AMEn and coefficient of apparent ileal digestibility (CAID) of nitrogen (N), fat, Ca and P. There was a significant ($P < 0.05$) interaction between nutrient density and feed form for CAID of DM and GE. Pelleting reduced ($P < 0.05$) the CAID of DM and GE only in M and VH diets and did not have effect ($P > 0.05$) on these parameters in other nutrient density diets. Feeding pellets lowered ($P < 0.05$) the CAID of N and starch. Pelleting significantly ($P < 0.001$) reduced the absolute weight of gizzard compared to mash diets. The gizzard pH of birds fed pellet diet was higher ($P < 0.001$) than those fed mash diets. In general, the current results show that the pellet-induced benefits on growth performance reduce as the nutrient density levels increase, highlighting the importance of considering nutrient density to maximise the benefits associated with pellet feeding.

Keywords: Broilers; Feed form; Nutrient density; Nutrient digestibility; Performance.

Acknowledgements

My sincere gratitude is expressed to my supervisor, Dr. M. Reza Abdollahi for his guidance, patience and commitment in conducting and writing this thesis.

I would also like to sincerely thank my co-supervisors, Professor Ravi Ravindran and Dr. Fifi Zaefarian for their guidance, understanding and encouragement during my study.

Am also thankful to the staff of the Massey University Poultry Research unit, Edward James, Colin Naftel, Shaun de Malmanche, Kalwyn Pereka and James Yamaguchi Hartley for helping my experimental work.

Special thanks to the Government of New Zealand through the Ministry of Foreign Affairs and Trade for the provision of the scholarship funding, so grateful for this support.

I am also thankful for the support received from the International Student Support team, Sylvia Hooker, Jamie Hooper, Logan Tate, Saba Azeem, Dianne Reilly and Tian Yang.

I would also like to sincerely thank my mother, sisters and brothers for their support and encouragement.

My earnest gratitude is expressed to my wife, Annie Banda, and daughters, Chuddy and Janel for their unconditional love, patience, support, understanding and encouragement and to whom this thesis is dedicated.

Above all, am so thankful to God Almighty for this achievement, the glory belongs to Him.

Table of contents

List of Tables

Chapter 3

List of Figures

Chapter 2

List of Abbreviations

AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
CAID	Coefficient of apparent ileal digestibility
Ca	Calcium
CP	Crude protein
d	Day
DM	Dry matter
F/G	Feed per unit gain
g	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GLM	General linear model
H	High
Kg	Kilogram
KJ	Kilo joule
L	Low
M	Medium
mg	Milligram
MJ	Mega joule
mm	Millimetre

N	Nitrogen
NSP	Non-starch polysaccharides
P	Phosphorous
PDI	Pellet durability index
Ti	Titanium
VH	Very high
VL	Very low

CHAPTER 1

General introduction

Dietary nutrient density is an important factor, which has significant impact on growth performance, carcass characteristics and economics of broiler production (Jackson et al., 1982; Jones and Wiseman, 1985). It has been reported that birds adjust feed intake to meet their energy requirements (Farrell et al., 1973; Maiorka et al., 2004). Several studies into dietary nutrient and energy density have shown that feeding dense diets resulted in efficient feed utilisation and an improvement in growth rate (Waldroup et al., 1976; Lott et al., 1992; Lesson et al., 1996b, 1999; Rosa et al., 2007). Therefore, it is important to establish optimal nutrient requirements of birds to maximise growth performance and profitability.

The other dietary factor in addition to nutrient density that has an effect on bird performance and feed efficiency is feed form (Abdollahi et al., 2018a). Nowadays, the majority of broiler feed is in crumble or pellet form, whereas mash diets are still fed in some countries where pelleting equipment is not available or considered uneconomical. Pelleting is the process in which small feed particles are agglomerated into larger particles through a mechanical process that involves moisture, heat and pressure (McEllhiney, 1985; Goodarzi Boroojeni et al., 2016). Since its introduction, pelleting has been practiced globally and became the most common hydro-thermal process in the preparation of broiler diets. Approximately eighty percent of monogastric feed in the US is pelleted (Behnke, 2001). The improvement in performance of meat-type chicken associated with pelleting has been attributed to factors such as: increased feed intake, reduced ingredient segregation, less time and energy spent during feeding, and reduced feed wastage (Bolton, 1960; Behnke, 2001; Peisker, 2006; Amerah et al., 2007b;).

Despite the higher growth performance associated with feeding pelleted diets compared to mash diets, poor development of proventriculus and gizzard has been observed (Nir et al., 1994; Amerah et al., 2007b). It has been suggested that inadequate mechanical stimulation associated with pelleted-diet is the cause of this negative effect of pelleting on upper digestive tract (Engberg et al., 2002). On the other hand, dietary composition has changed over the years from fibrous, textured and poorly digestible feed ingredients, to low fibre, texture-less and nutrient-enriched diets. This dietary transition may even worsen the effect of pelleting on upper digestive tract.

Inclusion of insoluble fibre in poultry diets helps improve the development and functionality of gut components as well as stimulating the production of hydrochloric acid in the proventriculus (Hetland et al., 2005; Gonzalez-Alvarado et al., 2007; Hetland et al., 2007). Fibrous materials act as a diluent, reduces the dietary energy and nutrient density (Mateos et al., 2012). Therefore, it is postulated that low nutrient density diets could maximise pelleting-induced benefits by overcoming the negative effect of pelleting on digestive tract development. Ingredients and feed cost and processing represent 60 -70 percent of the total production cost in poultry (Behnke, 1996; Abdollahi et al., 2013a), and ingredient cost constitutes the largest proportion (Agah and Norollahi, 2008; Goodarzi Boroojeni et al., 2016). Incorporation of fibre materials such as wheat bran and palm kernel meal, to some extent, can lower the cost of feed and maximise profit. Despite the potential for interactive effects between nutrient density and feed form in poultry, only few studies have investigated this possible interaction on broiler performance and nutrient digestibility (Lesson et al., 1999; Lecznieski et al., 2001; Lemme et al., 2006; Brickett et al., 2007; Abdollahi et al., 2018b) and none have examined the effects on nutrient digestibility in broilers at 35 d post-hatch.

Three chapters are covered in this thesis. General introduction is given in Chapter 1, while Chapter 2 covers the review of the existing literature on how growth performance, nutrient digestibility and gastro intestinal tract (GIT) development in broilers are affected by nutrient density and feed form. It also highlights the effect of nutrient density on pellet quality. The trial work is covered in Chapter 3 with aspects such as abstract, introduction, materials and method, results, discussion and conclusion.

The objectives of the experimental research presented in this thesis are,

a) To evaluate the influence of feed form and nutrient density, and their potential interactions on growth performance and nutrient and energy utilisation responses in broilers fed wheat-based diets.

b) To investigate the effect of nutrient density and feed form, and their potential interactions on GIT development and carcass yield in broilers at 35 d post-hatch.

CHAPTER 2

Literature Review

2.1 Introduction

Dietary nutrient density is one of the nutritional factors that affect voluntary feed intake of broilers (Jafarnejad et al., 2011). Feed intake is generally considered as the main driving factor for the improved animal performance, as the intake of nutrients is largely determined by feed intake (Abdelsamie et al., 1983; Abdollahi et al., 2018a). Therefore, nutrient density has a substantial effect on the growth of broilers as well as on the economic returns over feed costs (Jackson et al., 1982). The modern broilers are more responsive to feed intake stressors (Abdollahi et al., 2018a), hence, determination of optimal feed intake is crucial in achieving the desired bird performance (Jafarnejad et al., 2011).

Improved feed efficiency in poultry has been associated with an increase in dietary nutrient density (Guzman et al., 2015; Classen, 2016) and birds have a tendency of eating to satisfy their energy requirement (Leeson et al., 1996b). It has been reported that voluntary feed intake reduces as the dietary energy content increases (Veldkamp et al. 2005). Farrell et al. (1973) observed an inverse relation between feed intake and energy levels in broiler diets. Jackson et al. (1982) reported a reduction in feed intake with increasing dietary energy levels from 2600 to 3600 kcal ME/kg) in broilers (0-49 d) fed mash diet. Leeson et al. (1996b), observed similar energy intake per kg body weight regardless of the energy levels in the diets. They also reported that, increase in energy levels led to increase in protein intake per kg body weight because of protein and other nutrients being kept constant at each dietary energy level. In the study involving turkeys, Veldkamp et al. (2005) observed that increasing dietary energy levels in maize-based diets led to a reduction in feed intake and feed per unit gain (F/G), in a linear pattern.

Another dietary factor that has a major effect on feed intake is feed form (Svihus, 2014; Abdollahi et al., 2018b). Increased feed intake due to pelleting broiler diets has been reported to vary from 2.8 % (Serrano et al., 2012) to 64 % (Amerah et al, 2007b). Several studies suggested that higher feed intake increases weight gain and reduces the proportion of energy used for maintenance in relation to gain (Engberg et al., 2002; Amerah et al., 2007b; Jafarnejad et al., 2011;

Chewning et al., 2012). However, negative effect of pellet feeding on upper digestive including; proventriculus and gizzard has been observed in many studies (Chewning et al., 2012; Abdollahi et al., 2013b, 2018b), which attributed largely to insufficient mechanical stimulation associated with pelleted diets (Engberg et al., 2002). It has been reported that the inclusion of insoluble dietary fibre, especially of insoluble and lignified coarsely ground fibre, increases the grinding function of the gizzard and anti-peristaltic refluxes of the GIT, resulting in better mixing of digesta with digestive juices (Hetland et al., 2007; Mateos et al., 2012), therefore, the use of high fibre ingredients such as palm kernel meal or wheat bran would potentially resume gizzard functionality and gut integrity and health which is an issue with the feeding highly processed diets. However, identifying the optimum nutrient density to be used in pelleted diets need further applied research that consider issues of economy. Birds respond to fibre inclusion differently depending on; type of fibre source, level of inclusion, diet characteristics, also the physiological and health status of the birds (Mateos et al., 2012).

2.2 Feed forms for broilers (mash, crumble, pellet)

Feed form is crucial factor in broiler nutrition as it has a considerable influence on the growth performance and digestibility of nutrients (Massuquetto et al., 2018). Mash, crumble and pellet are the main feed forms in broiler production (Agah and Norollahi, 2008; Cerrate et al., 2009; Jafarnejad et al., 2011). Typically, mash is the feed form in which finely or coarse ground ingredients are mixed to have a complete balanced diet i.e. to provide well balanced feed for each mouthful (Agah and Norollahi, 2008; Jafarnejad et al., 2011). While pellets are obtained when mash diet is transformed through a mechanical process that uses pressure, heat and moisture (McEllhiney, 1985; Agah and Norollahi, 2008) and crumbles are achieved by reducing pellets to smaller sizes but coarser than mash (Jafarnejad et al., 2011), this is done to have the suitable feed particle sizes for the young birds which tend to have difficulties in ingesting larger particles (pellet) (Calet, 2007).

Generally, there has been a shift in terms of feed form used in broiler feeding, from a mash dominated system to crumble-pellet one. However, in some places broilers are still fed mash diet due to lack of pelleting equipment as well as pelleting process being considered uneconomical (Brickett et al., 2007). Mash diet has been perceived to yield better economical return mainly because of low mortality percentage associated with it (Agah and Norollahi, 2008; Jafarnejad et al., 2011), though low growth rate has been observed in various studies in comparison to pellet diet (Leeson et al., 1999).

In general, feed intake in birds fed pelleted-diet is higher than in those fed mash diet. Amerah et al. (2007b) reported that broilers fed wheat-based pelleted diet had 52.6 % higher feed intake compared to those fed mash diet. Abdollahi et al. (2011) found a significant difference in terms of feed intake, with pellet feeding having 13.7 % higher feed intake than mash one, in the study involving broilers fed wheat-based diet. Subsequently, Abdollahi et al. (2013b) reported a high intake of feed in favour of pellet feeding in broilers fed wheat- and maize-based diet. Improved feed consumption due to pellet feeding, was also reported in broilers fed sorghum-based diet during 21 d post-hatch (Abdollahi et al., 2014). However, Chewning et al. (2012) reported that the positive effect of pellet feeding on feed intake was only seen in grower and finisher phase in broilers fed maize-based diet but not in starter phase (0-14 d). Corzo et al. (2012) also reported the lack of feed form effect on feed intake during the early stage of life (0-18 d post-hatch) for broiler chicks fed maize-based diet.

Birds fed pelleted diet tend to spend less time and energy during feeding as a result productive energy is improved. Jensen et al. (1962) found that poults fed mash diet spent more time than those fed pelleted diet, 18.8 and 2.2 % of a 12-hour was spent feeding, respectively. Similarly, Savory (1974) reported that chicks (the hybrid and brown leghorn) fed pelleted diet spent less time feeding than those fed mash diet (re-ground pellets). The brown leghorn chicks spent 7.82 and 16.02 % of time feeding on pellet and mash diets, respectively. Abdelsamie et al. (1983) have not observed any significant difference in feed intake of the birds fed mash or pellet diets, but the difference was significant for weight gain and F/G in favour of pellet feeding.

Pelleting can have a large physical and chemical impact on feed components, including partial denaturation of protein and deactivation of anti-nutritional factors (Allred et al., 1957), and starch gelatinisation (Amerah et al., 2007b) which can cause an improvement in production efficiency. However, it should be noted that high heat and moisture applied during steam-conditioning may induce chemical changes which may be detrimental to nutrient availability and may reduce or completely negate the benefits of pelleting (Abdollahi et al., 2011; Moritz and Lilly, 2010).

2.3 Influence of feed form on broiler growth performance

Offering feed to poultry in pellet form enhances the economics of production by improving feed efficiency and growth performance in broilers (Behnke and Beyer, 2002). These improvements are attributed to decreased ingredient segregation and feed wastage, no selective feeding, higher bulk and nutrient density, decreased time and energy spent for eating, destruction of feed-borne pathogens, thermal modification of starch and protein, improved palatability and inactivation of enzyme inhibitors (Behnke, 1994; Jensen, 2000; Peisker, 2006). Allred et al. (1957) observed an improvement of 13.3 and 8.6 % in body weight and feed per unit again, respectively, of chicks (White Rock x White Olympian) fed pellet diet than those fed mash diet. Kilburn and Edwards (2001) reported that broilers fed maize-based pelleted diet had a better growth response than those fed mash diet. Likewise, Greenwood et al. (2005) found that pelleted diet (maize-based) improved body weight gain and F/G over mash diet during 16 to 30 d post-hatch. Improvement in body weight and F/G of broilers due to pellet feeding was also reported in other studies (Svihus et al., 2004; Kidd et al., 2005a; Jahan et al., 2006; Amerah et al., 2007b; Agah and Norollahi, 2008; Cerrate et al., 2009; Corzo et al., 2011; Jafarnejad et al., 2011; Chewning et al., 2012; Abdollahi et al., 2014; Naderinejad et al., 2016). In the study by Lemme et al. (2006), broilers fed maize-wheat-based pelleted diet had better body weight gain compared to those fed mash die during 14 to 35 d post-hatch, while F/G was better in broilers fed mash diet. However, Abdollahi et al. (2013b) reported lack of feed form effect on F/G in broilers fed either wheat- or maize-based diets. Similarly, Brickett et al. (2007) and Abdollahi et al. (2018b) found that F/G was not statistically different between broilers fed pelleted and mash diets.

The effect of feed form has also been investigated in pullet/layer-chickens. Frikha et al. (2009) reported that feed intake and body weight gain were higher in pullets fed pelleted wheat-maize-based diet compared to those fed mash diet. Further, Guzman et al. (2015) reported that pellet feeding improved the average daily gain (ADG) by 6.5 % and F/G by 8.2 % but lowered the average daily feed intake (ADFI) by 1.9 % for pullets (0-5 weeks of life) fed maize-wheat-based diets. Saldana et al. (2015a, b), also observed an increase in feed intake, improvement in body weight gain and F/G due to pellet feeding in pullets reared for seventeen weeks post-hatch. The positive effect of pellet compared to mash diets on growth performance has also been observed in turkeys (Blakely et al., 1963).

2.4 Influence of feed form on nutrient digestibility

2.4.1 Starch

Starch is the storage component of carbohydrate in grains (Moran, 1982; Knudsen, 1997), and is the main source of energy in broiler diets (Choct, 2009), comprising approximately 40% of the diet and contributing to more than half the metabolisable energy intake (Svihus, 2011). Starch is a glucan that consist of amylopectin and amylose, in helical and linear form and the ratio of 75:25, respectively (Moran, 1982; Knudsen, 1997; Carre, 2004; Abdollahi and Ravindran, 2012).

The integrity of amylopectin and amylose is destabilised during processing (pelleting) when exposed to heat and water that causes swelling (crystallinity is lost) of the granules (Moran, 1982). Absorption of water by granules initiates gelatinisation. This process is concluded with the release of amylopectin and amylose molecules when heat is supplied to the swelled granules (Moran, 1982; Zaefarian et al., 2016). This swelling of granules become irreversible at a certain temperature, and significant alteration of the granules occurs (Abdollahi and Ravindran, 2012). When the gelatinised starch-water mixture is cooled to room temperature, the process called retrogradation occurs which involves the re-assembling of starch molecules to original state that existed before gelatinisation process (Abdollahi and Ravindran, 2012). Digestibility of starch is improved due to alteration of granules which occurs through thermo-mechanical treatment (Carre, 2004). Therefore, the digestion of starch in the GIT tend to vary depending on chemical and physical (feed form) characteristics of the diet, as well as the cereal used in the diet. Zimonja and Svihus (2009) reported that starch gelatinisation was lower in birds fed wheat-based diet compared to birds fed oat-based diet. In their study, starch digestibility increased in extruded compared to steam or cold-pelleted wheat-based diets, due to an increase in starch gelatinisation caused by extrusion process.

Abdollahi et al. (2013b) reported that pelleting lowered the coefficient ileal apparent digestibility (CIAD) of starch in wheat-based diets compared to mash feeding. Likewise, Abdollahi et al. (2014) observed a significant reduction in starch digestibility due to pellet feeding in broilers fed red sorghum-based diet when compared to those fed mash diet. Subsequently, Abdollahi et al. (2018b) observed the negative effect of pellet feeding on CIAD of starch in broilers fed maize-based diet compared to mash diet. However, Abdollahi et al. (2013b) found that feed form had no significant effect on CIAD of starch in broilers fed maize-based diet. The same results have been reported by Massuquetto et al. (2018), who observed the lack of feed form effect on starch digestibility in broilers fed maize-based diet in the early stage of life (0-25 d).

Svihus and Hetland (2001) attributed the low starch digestibility in wheat-based diets to increased load of starch in the gut due to high feed intake. Generally, there is an inverse correlation between starch digestibility and feed intake, also, high feed intake tends to pose a physiological limitation in the modern broilers resulting in suboptimal starch digestion (Svihus, 2011). The other suggested reason is related to cell wall structure and hardness characteristics of wheat which allows to some extent the entrapped starch granules to remain in the cell wall of aleurone layer and protein matrix (Svihus, 2011).

2.4.2 Protein and amino acids

The effect of feed form on digestion of protein or amino acids also tend to vary based on cereal used in the diet. For example, Abdollahi et al. (2011) observed a reduction in the digestion of nitrogen (N) in broilers fed wheat-based pelleted diet compared to those fed mash diet. However, Abdollahi et al. (2013b) observed no effect of feed form on CIAD of N in broilers fed maize-based, while in birds fed wheat-based diet, pelleting reduced CIAD of N compared to mash diet. Naderinejad et al. (2016) also indicated the reduction of CIAD of N due to pellet feeding in broilers fed maize-based diet in comparison to those fed mash diet.

In the recent study, Massuquetto et al. (2018) reported a higher CIAD of N in broilers fed maize-based pelleted diet when compared to those fed mash diet. In contrast, Abdollahi et al. (2018b) reported that pelleting lowered the CIAD of N compared to mash feeding in broilers fed maize-based diet.

2.4.3 Fat

Effect of feed form on digestibility of fat is variable based on cereal source used in the study. Abdollahi et al. (2013b) reported that pellet feeding improved the CIAD of fat by 10.9 % compared to mash feeding in broilers fed maize-based diet, while in birds fed wheat-based diet, the CIAD of fat was reduced by 13.7 % due to pelleting in comparison to mash diet. In the subsequent study, Abdollahi et al. (2014) observed a reduction in CIAD of fat in birds fed sorghum-based pelleted diets. Moreover, Abdollahi et al. (2018b) reported that feeding pellet lowered CIAD of fat compared to mash in broilers fed maize-based diet at 21 d post-hatch.

2.4.4 Calcium and phosphorus

The digestibility of calcium (Ca) and phosphorus (P) also tend to vary based on cereal source in the diet. Kilburn and Edwards (2001) reported that phytate P retention significantly reduced in broilers fed maize-based pelleted diet compared to those fed mash diet. However, Abdollahi et al. (2013b) reported high CIAD of Ca and P in birds fed pelleted maize-based diet, compared to those fed mash diets, while for the wheat-based diet, pelleting had negative effect on these parameters. In the following study, Abdollahi et al. (2014) observed a reduction in CIAD of Ca due to pelleting in broilers fed sorghum-based diet, but no feed form effect was observed on CIAD of P. Naderinejad et al. (2016) observed that the CIAD of Ca and P was reduced due to pelleting in comparison to mash in broilers fed maize-based diet.

2.4.5 Metabolisable energy

Amerah et al. (2007b) reported that the nitrogen-corrected apparent metabolisable energy (AMEn) was lowered in broilers fed wheat-based pelleted diet compared to those fed mash diet. Abdollahi et al. (2011; 2013b) also observed the reduction in AMEn due to pellet feeding in comparison to mash feeding in broilers fed wheat-based diet. Similar results were observed in the study by Abdollahi et al. (2014) with sorghum-based diets in 21 d post-hatch.

However, Svihus et al. (2004) reported a significant increase in AME of pelleted wheat-based diet compared to mash diet (11.6 to 11.8 MJ/kg, respectively). Further, the effect of feed form on AMEn was not significant in broilers fed maize-based diet (Abdollahi et al., 2013b). A higher ileal digestible energy (IDE) was reported in birds fed maize-based pelleted diet compared to those fed mash diet, in Massuquetto et al. (2018) study. Conversely, Abdollahi et al. (2018b) reported that pelleting reduced AMEn compared to mash in broilers fed maize-based diet at 21 d-of age.

2.5 Influence of feed form on digestive tract functionality

2.5.1 Effect of pelleting on feed particle size

Feed particle size is important in achieving desired growth performance of birds, as it has a considerable influence on the development of gut components such as the proventriculus and gizzard (Zaefarian et al., 2016). Generally, feed particle size tends to be evened out by pelleting process (Svihus et al., 2004; Amerah et al., 2007a, 2007b; Abdollahi et al., 2018a), therefore, particle size influence in pellet is to some extent less important compared to mash diet (Amerah et al., 2007a). Kilburn and Edwards (2001) reported that in mash diets the coarse maize (2897 µm) particles showed a significant improvement in Ca and phytate P retention compared to the fine particle (869 µm), however, this improvement disappeared with pelleting of these diets. Engberg et al. (2002) observed a reduction in feed particle size due to pelleting, the differences between the finely and coarsely ground pellets was evened up. In addition, Svihus et al. (2004) observed a considerable increase in the ratio of fine particles (< 0.2 mm in size) when wet sieving was used for pelleted wheat-based diet. This trend was also observed in Amerah et al. (2007b) study. They found that wheat particle sizes (3.0 mm and 7.0 mm) had no effect on broiler performance in pelleted diet, while in mash diet, feed intake, F/G and body weight gain improved in coarse (7.0 mm) than in the medium particle (3 mm). In addition, Naderinejad et al. (2016) reported that different particle sizes (fine, medium and coarse; 2.0, 5.0 and 8.0 mm, respectively) in pellet diet did not have a significant effect on broiler parameters, however, starch digestibility and AMEn were affected by particle size in pellet diet with coarse particles having the highest of these parameters.

2.5.2 Effect of pelleting on upper gut development

The gizzard and proventriculus are important digestive tract components and adequate development and functionality of these components is of great importance in having a functional GIT (Mateos et al., 2012). Gizzard is known to influence physiological aspects of GIT such as; motility control, reducing feed particle size, regulate gastroduodenal refluxes and flow of feed, and enhancement of endogenous enzymes, bile acids and hydrochloric acid (HCl) secretion (Mateos et al., 2012). Generally, enhancement in nutrient digestion due to greater motility and grinding is associated with a well-developed gizzard (Amerah et al., 2007a). Because of this realisation, several studies have been conducted to establish strategies to improve proventriculus and gizzard development.

Feed form is suggested to have a considerable influence on functionality of digestive tract which in turn affect bird performance (Svihus, 2014). Pellet feeding has generally been associated with poor development of gizzard mostly due to inadequate feed mechanical stimulation (Engberg et al., 2002). The reduction in gizzard weight was observed in the study by Abdelsamie et al. (1983) involving broilers fed cassava-sorghum-based diet for 56 d post-hatch. Nir et al. (1994) reported that pellet feeding reduced the relative weight of gizzard when compared to mash feeding in broilers fed maize- or sorghum-based diet. They also reported that pelleting lowered activity of amylase in intestinal content, and trypsin in the pancreas. Engberg et al. (2002) observed a significant reduction in gizzard relative weight, and an increase in gizzard pH associated with pellet feeding. In Svihus et al. (2004) study, pelleting reduced the content and relative weight of gizzard of broilers fed wheat-based diet. Similarly, Amerah et al. (2007b) reported a reduction in proventriculus and gizzard relative weight in broilers fed pelleted wheat-based diet when compared to those fed mash diet.

This trend has also been observed in several other studies. Abdollahi et al. (2011) reported a reduction in relative weight of proventriculus and gizzard in broilers fed wheat-based pelleted diet compared to those fed mash diet. Chewning et al. (2012) reported a reduction in absolute and relative gizzard weights in broilers fed pelleted maize-based diet compared to those fed mash diet. In the study by Abdollahi et al. (2013b) pellet feeding reduced relative weight of gizzard in both wheat and maize-based diets compared to mash feeding. Similarly, Naderinejad et al. (2016) and

Abdollahi et al. (2018b) observed a reduction in relative weight of proventriculus and gizzard in broilers fed pelleted maize-based diet than those fed mash diet.

Effect of pelleting on upper gut development has also been investigated in pullets. Frikha et al. (2009) reported that pellet feeding reduced relative weight of gizzard and proventriculus in pullets at 45 d of age when compared to mash feeding. Similar results were observed in Saldana et al. (2015a) study. They found that relative weight of gizzard was lower in pullets fed crumbled than those fed mash diets at 5, 10 and 17 weeks of post-hatch. Furthermore, Saldana et al. (2015b) reported that pellet feeding in comparison to mash feeding led to higher gizzard pH and lower gizzard weight in pullets fed maize or wheat-based diet at 17 d of age.

2.5.3 Effect of pelleting on small intestine and caeca

The small intestine (duodenum, jejunum and ileum) is one of the important segments of the digestive tract for digestion and absorption of nutrients (McDonald, 2011), and the caeca for absorption of water and electrolyte (Svihus, 2014). Feed form affects the development and functionality of gut components (Svihus, 2014). Nir et al. (1994) reported that the length of ileum and jejunum reduced due to pelleting in the meat-type chicken. In the study by Amerah et al. (2007b) pelleting reduced the length of small intestine. They reported that there was an increase in mucosal layer (it tends to vary with respect to the flow of nutrients), villus height and crypt depth of duodenum and jejunum of birds fed pelleted diet compared to birds fed mash diet. These improvements are important in enhancing digestion and absorption of nutrients in small intestine (Amerah et al., 2007b). Abdollahi et al. (2011) observed a reduction in relative weight of caeca in pellet fed compared to mash fed broilers. Abdollahi et al. (2013b) also reported that feeding pellets reduced relative length (cm/kg body weight) as well as relative weight (g/kg body weight) of small intestine in wheat and maize-based diets compared to feeding mash diets. Similarly, a reduction in relative weight (g/kg feed intake) of duodenum, jejunum and ileum due to pellet feeding was reported in Abdollahi et al. (2018b) study in broilers fed maize-based diet in 21 d post-hatch.

Similar results have been observed in Frikha et al. (2009) study on pullets, they found that feeding pellets to 45 d old pullets reduced the relative length of small intestines and caeca.

2.5.4 Effect of pelleting on microbial population in the digestive tract

The GIT harbours several microbes, for example, more than 640 different bacteria species are known to exist in the gut (Apajalahti et al., 2004). For poultry, the small intestine is generally dominated by lactic acid bacteria, especially the lactobacilli (Engberg et al., 2002). These bacteria species found in the digestive tract act on different substrates and have different growth requirements. In addition, the slowly absorbed and resistant dietary components to digestive fluids are the main energy source for these bacteria (Apajalahti et al., 2004). Because of this, the distribution of the bacteria in the gut is mostly influenced by the digesta in terms of its structure and chemical composition (Apajalahti et al., 2004). Generally, diet (physical and chemical changes) has a considerable influence on population and distribution of microbes in the gut (Apajalahti et al., 2004; Choct, 2009).

In a broiler study, Engberg et al. (2002) reported that pellet feeding increased coliform bacteria and enterococci population in the ileum but reduced that of lactobacilli and *Clostridium perfringens* in the caeca and rectum.

2.6 Influence of feed form on mortality and leg abnormalities

Ascites, sudden death syndrome (SDS) and skeletal problems/mortalities are increasing due to high growth rate in meat-type chicken, (Leeson et al., 1999; Engberg et al., 2002). Nir et al. (1995) observed an increase in mortality in broilers fed pelleted maize-based diet compared to those fed mash diet. Leeson et al. (1999) reported that mash feeding reduced mortality compared to pellet feeding, (3.9 *vs.* 15.3 %, respectively), in broilers fed maize-based diet. Lecznieski et al. (2001) also observed mortality of 8.3 and 17.8 % in broilers fed mash and pellet maize-based diets, respectively. Further, Brickett et al. (2007) reported that mortality was high in broilers fed pelleted barley-maize-wheat-based diet than those fed mash diet, (5.6 *vs.* 3.8 %, respectively). Other studies have also reported the increase in mortality due to pelleting compared to the mash, in maize-based (Kidd et al., 2005a; Agah and Norollahi, 2008) or wheat-based (Engberg et al., 2002) diets.

However, early study by Abdelsamie et al. (1983), showed that feed form had no effect on mortality in broilers fed maize-based diet. The lack of feed form effect on mortality in broilers was also reported in other studies (Scott, 2002; Cerrate et al., 2009; Corzo et al., 2011; Corzo et al., 2012).

2.7 Effect of nutrient density on broiler growth performance

Dietary nutrient density has a substantial effect on bird growth performance. Holsheimer and Ruesink (1993) reported an improvement in F/G and body weight gain with increasing lysine levels (0.97, 1.06 and 1.15 %) in broiler starter diet (0-14 d). Similar results were reported by Temim et al. (2000), with crude protein (CP) level increasing from 10 to 15, 20, 28 and 33 % in broiler diet. Lemme et al. (2006) observed an increase in weight gain, improvement in feed efficiency and a reduction in feed intake with the increasing the protein ideal balance (9.7, 10.7, 11.7 and 12.7 g digestible lysine/kg of diet) in maize-wheat-based diet in broilers. The improvement in F/G due to the increase in nutrient density was also observed in the study conducted by Brickett et al. (2007), in which broilers were fed barley- or wheat- and maize-based diet. Jafarnejad et al. (2011) found that the change in dietary protein from 21 to 23 % led to a significant improvement in F/G and body weight in broilers fed maize-based diet. Lilly et al. (2011) investigated the effect of different levels of amino acid in pelleted finisher (28-42 d) diet on growth performance of broiler. They observed a significant improvement in F/G and body weight as the dietary amino acid density increased. Similarly, Basurco et al. (2015) reported an improvement in body weight and F/G and reduction in feed intake as the ideal protein balance increased in maize-based diet during 29 d post-hatch.

Similarly, dietary energy manipulation has a considerable effect on bird growth performance. For example, Jackson et al. (1982) observed a significant improvement in body weight and F/G as the dietary energy levels increased by 200 kcal/kg from 2600 to 3600 kcal ME/kg. Jones and Wiseman (1985) also investigated the influence of dietary energy levels (2575, 3053 and 3530 kcal ME/kg) on broiler performance. They observed that birds fed high-energy starter diet were heavier than those fed low-energy diet. Further, Holsheimer and Veerkamp (1992) reported that broilers fed maize-pelleted diet containing high-dietary energy (3200 kcal ME/kg) performed better than those fed low-energy (2880 kcal ME/kg) diets in terms of body weight gain as well as F/G. However, this is in contrast with the result of Skinner et al. (1992), who reported a significant reduction in body weight gain and feed intake of broilers with increasing dietary nutrient density from 3080 to 3465 kcal ME/kg during 42 to 49 d of age. Nevertheless, Nawaz et al. (2006) observed a significant increase in feed intake and body weight gain in broilers fed diet low in energy (2800 and 3000 kcal ME/kg; starter and finisher phase, respectively) compared to those fed high energy diet (3000 and 3200 kcal ME/kg; starter and finisher phase, respectively). Rosa et al. (2007) reported that increase in dietary energy level (2950 to 3450 kcal ME/kg) had no effect on F/G but weight gain increased significantly by increasing energy level in the diet. The improvement in growth performance of meat-type chicken associated with high-energy diets have also been reported in other studies (Waldroup et al., 1976; Maiorka et al., 2004).

Leeson et al. (1996b) found lack of significant effect of different energy concentrations (2700, 2900, 3100 and 3300 kcal ME/kg) on body weight gain in broilers fed maize-based diet, however, a reduction in feed intake and improvement in F/G were observed as the dietary energy level increased. Similarly, Lecznieski et al. (2001) found that different dietary energy levels had no effect on body weight gain in broilers, but the improvement in F/G was observed with the increase in energy levels.

The effect of dietary energy levels in pullet/layer performance are variable. Frikha et al. (2009) observed an improvement in body weight gain and F/G of pullet as the energy level of diet increased from; 2761 to 2801 and 2880 kcal ME/kg. In addition, Saldana et al. (2015a) reported that the increase in energy content (2850 to 3050 kcal ME/kg, differed by 50 kcal ME/kg) in wheat- maize- and barley-based diet fed to pullets at 17 weeks post-hatch significantly improved the F/G but lowered the daily feed intake. Similarly, Saldana et al. (2016) observed an improvement of 3.1 % in utilisation of feed per kilogram of eggs, and 3.7 % reduction in average daily feed intake as the dietary energy increased from 2653 to 2753 kcal ME/kg in layers at 17-46 weeks of age. However, these changes in energy content had no effect on egg productivity.

2.8 Effect of nutrient density on nutrient digestibility

2.8.1 Starch

The digestion of starch is affected by nutrient density. Abdollahi et al. (2018b) showed that broilers fed very low-density diet had higher starch digestibility compared to those fed very high nutrient density diet. They associated the low starch digestibility to poor developed of the gizzard observed in their study; relative gizzard weight reduced as the nutrient density increased.

2.8.2 Protein and amino acids

Cowieson and Ravindran (2008) reported that nutrient density (varying by 0.63 MJ/kg AME, and 3 % in amino acid levels) had no effect on the digestibility of N in broiler starters fed mash maize-based diet. While, Abdollahi et al. (2018b) reported that an increase in nutrient density led to a significant improvement in the digestibility of N from 0.772 (very low nutrient density diet) to 0.811 (very high nutrient density) in broilers fed maize-based diet during 21 d post-hatch.

2.8.3 Fat

Kalmendal et al. (2011) observed a significant improvement of 5.8 % in fat digestibility as dietary energy reduced from 2890 to 2723 kcal/kg in pelleted maize-based diet fed to broilers during 15 to 31 d of age. However, Abdollahi et al. (2018b) found that nutrient density had no effect on fat digestibility.

2.8.4 Calcium and phosphorus

Variable results have been reported regarding the effect of nutrient density on Ca and P digestibility. Cowieson and Ravindran (2008) reported that different nutrient concentration had no effect on Ca and P digestibility in broilers at 21 d post-hatch. But, Abdollahi et al. (2018b) found that broilers fed very low nutrient density diet had higher CIAD of Ca compared to those fed very high nutrient density diet. They also observed that P digestibility was higher in birds fed very high-density diet than those fed very low-density diet.

2.9 Influence of nutrient density on gastro intestinal tract development

Nawaz et al. (2006) showed that varying dietary energy levels in broilers (2800 - 3000 and 3000 - 3200 ME kcal/kg in starter and finisher, respectively) did not influence the absolute weight of gizzard. Abdollahi et al. (2018b) found that the absolute weight of proventriculus and gizzard were not affected by the changes in nutrient density, but the absolute weight of small intestine segments increased with the increase in nutrient concentration in broilers fed maize-based diet during the starter period. They also reported that relative weight (g/kg feed intake) of proventriculus, duodenum, jejunum and ileum increased with increasing nutrient density with exception of gizzard which relative weight reduced.

In pullet and layers the effect of nutrient density on GIT development is variable. Frikha et al. (2009) reported that relative weight (g/kg body weight) of all digestive tract components reduced with increasing level in dietary energy (2761, 2801, and 2880 kcal ME/kg) in 46 d old pullets. However, the relative length (cm/kg body weight) of these segments was not affected by the changes in dietary energy level. Saldana et al. (2016) reported the lack of effect of energy concentration on relative weight of gizzard, small intestine, cecum as well as gizzard pH in layers fed maize- wheat- and barley-based diet during17 - 46 weeks of age.

2.10 Influence of nutrient density on mortality and leg abnormalities

Skinner et al. (1992) reported lack of effect by nutrient density on mortality of broilers during 42 to 49 d of life, when energy was increased by 55 ME kcal/kg from 3080 to 3465 ME kcal/kg in maize-based diet. Kidd et al. (2005b) found that changes in ileal digestible amino acids with respect to lysine ratio in maize-based diet had no significant effect on mortality of broilers reared for 55 d post-hatch. In the study by Brickett et al. (2007) in which broilers were fed barley-maize-based diet for 35 d, the effect of nutrient density was not significant for mortality. Similarly, the lack of significant effect of different lysine levels in maize-based diet on mortality of broilers was reported in Corzo et al. (2012) study. Same results were observed by Holsheimer and Ruesink (1993).

In contrast to these results, Farrell et al. (1973) observed a significant effect of dietary energy concentration (2300 to 3600 kcal ME/kg) on broiler mortality. They reported that mortality was low in birds fed medium energy level (3100 kcal/kg) in their diet compared to other energy levels. Scott (2002) reported that mortality increased from 0.72 to 1.81 % when birds were fed high-density diet. In this study ME level for starter diet was from 3100 to 3170 kcal/kg and protein level was from 235 to 251 g/kg, and for grower diet, ME level was from 3060 to 3200 kcal/kg and protein level was from 195 to 210 g/kg.

2.11 Feed form and nutrient density interaction on growth performance and nutrient utilisation

Allred et al. (1957) observed no significant interaction between protein levels and pelleting in poults fed maize-based diet. They reported that different CP levels (20, 22 and 24 %) did not change the effect of pelleting on F/G and growth response. Lemme et al. (2006) reported lack of interaction between ideal balanced protein and feed form for feed intake, weight gain and F/G in broilers fed maize-wheat-based diet from 14 to 35 d. Similarly, Jafarnejad et al. (2011) observed no interaction between protein levels and feed form, but for energy density this interaction was significant in broiler starters fed maize-based diets.

Greenwood et al. (2005) found significant interaction between feed form and digestible lysine for body weight gain and F/G in broilers fed maize-based diets during 16 to 30 d post-hatch. Brickett et al. (2007) found that feed intake was high in broilers fed low-density compared to those fed high-density pellet diets, but this trend was not observed in mash diets. Further, Corzo et al. (2012) reported of an interaction between feed form and digestible lysine (0.85, 0.95, 1.05, 1.15 and 1.35 %) for body weight gain and F/G in broilers fed maize-based diets. They found that birds fed mash diet attained body weight and F/G like birds fed pelleted diet only at the highest lysine level. Abdollahi et al. (2018b) observed a significant interaction between nutrient concentration and feed form for feed intake, weight gain and F/G. The interaction was also significant for CIAD of Ca and P. However, this interaction was not significant for CIAD of N, starch, fat and AMEn, as well as for absolute weight of proventriculus, gizzard and small intestine segments.

Effect of nutrient density and feed form interaction has also been investigated in pullets. Guzman et al. (2015) observed a significant interaction between feed form and energy density for ADFI and F/G in pullets fed maize-wheat based diet for 5 weeks post-hatch. They also found that the energy intake was similar regardless of the energy concentration in crumbled diet, however, in mash diet the energy intake increased as the energy concentration increased.

2.12 Effect of feed form and nutrient density on carcass characteristics

Nutrient density is one of important factors influencing broiler production parameters and carcass yield, which consequently affects profitability (Basurco et al., 2015). Hence, this aspect requires considerable attention. Bartov et al. (1974) observed an increase or decrease in carcass fat when dietary protein levels were decreased or increased, respectively. Jones and Wiseman (1985) found that the total carcass and abdominal fat were low in birds fed low-energy compared to those fed high-energy diet during the starter phase (0-24 d). Shen et al. (1985) stated that the increase in dietary energy concentration led to an increase in carcass fat in broiler fed maize- or barley-based diet. Holsheimer and Veerkamp (1992) also observed an increase in carcass fat with increasing the energy content of diets of broilers. Moreover, Corzo et al. (2007) reported that absolute weight of carcass, abdominal fat and breast meat increased as the dietary threonine increased in maize-based diet. Rosa et al. (2007) also reported an increase in abdominal fat pad with increasing dietary energy levels, but no effect on carcass, breast, wings, thighs and drumsticks weights. Other studies (Waldroup et al., 1976; Mabray and Waldroup, 1981; Holsheimer and Ruesink, 1993; Wiseman and Lewis, 1998; Temim et al., 2000) also reported the positive effect of increasing dietary nutrient and energy on carcass characteristics.

Leeson et al. (1999) found that energy density had no effect on carcass and breast weight, but the effect was significant for absolute and relative weight of abdominal fat pad; with increasing energy levels, these parameters increased. Nawaz et al. (2006) showed that nutrient density had no effect on abdominal fat percentage in broilers fed maize-wheat-based diet. Lilly et al. (2011) reported that an increase in amino acid density of pelleted maize-based diet in broilers did not have a significant effect on thigh and carcass yield during 28 to 42 d post-hatch.

In contrast, Skinner et al. (1992) reported that the increase in energy levels in maize-based diet led to a significant reduction in abdominal fat pad in broilers at 42 to 49 d of life. Similarly, Leeson et al. (1996a) observed a reduction in abdominal fat when the dietary energy levels was reduced by 250 kcal ME/kg at 35 to 49 d of age. Lemme et al. (2006) also observed a decrease in abdominal fat with the increase in balanced protein in broilers. Further, Veldkamp et al. (2005) reported that carcass and breast meat yields reduced significantly as the dietary energy content of diets fed to turkeys increased.

The effect of feed form on carcass characteristics is also variable. Leeson et al. (1999) reported that absolute carcass weight was high in broilers fed pelleted diet compared to those fed mash diet. Lecknieski et al. (2001) observed an increase in abdominal fat pad in broilers fed pellet compared to those fed mash diets during 22 to 43 d post-hatch. The positive effect of pelleting on carcass weight compared to mash diet was also reported in Kidd et al. (2005a), Lemme et al. (2006) and Corzo et al. (2011) studies. Saldana et al. (2015a) reported that pellet feeding in pullets increased carcass weight compared to mash feeding.

However, Kidd et al. (2005a) showed that feed form had no significant effect on the abdominal fat pad in broilers fed maize-based diet. Similarly, Agah and Norollahi, (2008) observed that feed form (pellet, crumble and mash) had no effect on carcass, breast, thigh and abdominal fat pad weights in broilers fed maize-based diet during finisher phase.

2.13 Woody breast and white striping

The attention to myopathy (muscle disease; fibre muscle not functioning normally) especially the woody breast and white striping of chicken-meat is increasing globally (Tijare et al., 2016). For example, under commercial condition in Italy, it is estimated that above 40 % of breast meat from medium and heavy chickens is affected by incidences of white striping (Lorenzi et al., 2014). In addition, acceptance of chicken-meat with these conditions generally is poor and this tend to have a considerable negative impact on the economics of production (Kuttappan et al., 2012c, 2016). In the study by Kuttappan et al. (2012c), it was shown that consumer acceptance was lowest for broiler fillets with severe score of white striping, and the level of dislike by consumers were; 10.7, 22.4 and 56.7 % for normal, moderate and severe score, respectively. Over 50 % of consumers showed no interest in buying fillets with moderate or severe score of white striping. The meat quality particularly water holding capacity is also negatively affected by woody breast and white striping conditions (Tijare et al., 2016). This aspect was further demonstrated in the study by

Tricino et al. (2015) in which cooking losses of 25.6 and 22.1 % in wooden and normal breast fillets was observed, respectively.

Generally, white striations running parallel with muscle fibres on thighs, breast, and other tender muscles characterise the white striping condition (Russo et al., 2015; Kuttappan et al., 2016), (Figure 2.1). While consistency toughness of the raw breast broilers characterises woody breast condition (Figure 2.2; Kuttappan et al., 2016). In terms of histology, these conditions are mostly characterised with fibrosis, myodegenaration, lipidosis, necrosis as well as regenerative changes (Petracci et al., 2013).

Figure 2. 1: Intensity (scores) of white striations in broiler fillets. Source: Kuttappan et al. (2016)

The scores (0 – 3) in the figure above, shows the different levels of White Striping condition in breast fillet; 0 = normal, 1 = moderate, 2 = severe, and 3 = extreme. Normal – no distinct white lines, moderate – small white lines (< 1 mm thick), severe – large white lines (1 -2 mm thick), and extreme – thick white bands (> 2 mm thickness).

Figure 2. 2: **Severe woody breast (WB) *vs.* normal breast fillet of chicken. Source: Kuttappan et al. (2016).**

The actual cause of woody breast and white striping is not yet fully established, however, aspects like rapid-growth rate in meat-type chicken is suggested to be the cause (Kuttappan et al., 2016). Occurrence of white striping tends to be high in heavy birds and is strongly related to average daily gain and body weight of birds (Russo et al., 2015). In addition, woody breast tends to be more severe in male than female broilers (Trocino et al., 2015), and the intensity of white striping generally increases as the breast weight or meat yield increases (Kuttappan et al., 2012b). In the study by Kuttappan et al. (2012b), broilers (processed at d 54) fed a low-energy (3000 kcal/kg) diet had a normal score in terms of white striping, while chicken fed a high-energy (3200 kcal/kg) diet had a more severe score and degree of white striping. Lorenzi et al. (2014) reported that the incidence of white striping was more severe in high-breast yield compared to the standard-breast yield of meat-type chicken, having slaughter weights of 3.8 - 4.2 and 3.0 - 3.8 kg, respectively, at the same age. To reduce the incidence of white striping, these researchers suggested that growth rate of meat-type chicken should be reduced. These results agree with those observed in the study by Russo et al. (2015) in which white striping severity percentage of 13.3 and 25.7 in medium (2.59 kg) and heavy (3.64 kg) weight broiler was found, respectively. They further suggested body weight to be among the predisposing factors for the occurrence of white striping in broilers. Further, Tijare et al. (2016) observed that the increase in woody breast and white striping tends to increase sarcomere length of broiler fillets.

2.14 Relevance of soluble fibre in broiler diets

The cell wall of plant material is mainly made of lignin and non-starch polysaccharides (cellulose and hemicellulose) usually known as dietary fibre (Knudsen, 1997; McDonald, 2011). The non-starch polysaccharides (NSP) are normally considered into two categories, insoluble and soluble NSP (Amirkolaie et al., 2005). Arabinoxylan, ß-glucan and pectin are some of the examples of soluble NSP (Choct, 1997; Hetland et al., 2007). Wheat, barley, and citrus pulp are rich in these soluble NSPs: arabinoxylan, ß-glucan and pectin, respectively (Liu et al., 2011). Soluble fibre is associated with high viscosity compared to insoluble fibre in the digestive tract, hence, it tends to affect the performance of birds negatively (Choct et al., 1996; McDonald, 2011). Generally, the digesta transit time is slowed by the viscosity and this to some extent encourages the fermentative microorganisms to proliferate in the small intestine of birds, hence, affecting the digestion and absorption of nutrients in the GIT (Choct et al., 1996). Langhout et al. (1999) observed a significant increase in the activity of microbes in ileum, especially the *Clostridia, E. coli, Enterococci,* and *Bacteroidaceae* in broiler chickens fed a diet containing high-methylated citrus pectin compared to those fed a diet low in pectin.

Annison (1991) observed the negative correlation between arabinoxylan (NSP of wheat) and apparent metabolisable energy. In addition, Choct et al. (1996) reported that ileal digestibility of protein, starch and fat reduced by 33.9, 37.4 and 59.0 %, respectively, when soluble NSP extracted from wheat was added to a commercial broiler diet. This negative effect of soluble NSP on nutrient digestibility was also observed in the study by Langhout et al. (1999), who reported that the inclusion of high-methylated citrus pectin in a broiler diet significantly reduced the digestibility of starch, crude fat, and amino acids. These researchers also observed a reduction in weight gain and feed efficiency in birds fed diet containing high-methylated citrus pectin.

2.15 Relevance of insoluble fibre in broiler diets

Cellulose (e.g. oat hulls) is one of the examples of insoluble NSP (Hetland and Svihus, 2001). Generally, insoluble fibre has been used a nutrient diluent in broiler nutrition (Choct, 2009; Mateos et al., 2012). The optimal fibre level in broiler diets have been found to have a positive effect and be useful; it enhances enzyme secretions, improves development and functionality of GIT components as well as increases gastroduodenal refluxes, which collectively improves digestibility of nutrients (Mateos et al., 2012). Structural components are essential in proper development and functionality of the gizzard, and the optimal level of dietary fibre helps in controlling the passage of digesta as well as nutrient digestion in the digestive tract (Hetland et al., 2007). Hetland et al. (2005) reported a 60 % increase in gizzard weight (including digesta) in layers given access to wood shavings compared to those that had no access to additional fibre (wood shavings). Similarly, Amerah et al. (2009) indicated that the inclusion of wood shavings in a wheat-based diet increased the relative weight of gizzard as well as the digestibility of ileal starch in broilers (21 d of life).

The fibre also tends to increase feed retention time and reduce the pH in the gizzard in broilers (Abdelsamie et al., 1983; Svihus, 2014). Gonzalez-Alvarado et al. (2007) reported that the inclusion of oat and soy hulls in broiler starter diets improved the total tract apparent retention of nutrients. Gonzalez-Alvarado et al. (2010) also observed the improvement in total tract retention of DM, fat and N when oat hulls were incorporated into the diet of broilers at 42 d post-hatch. Guzman et al. (2015) investigated the effect of fibre (cereal straw, sugar beet pulp and sunflower hulls) on the performance of pullets (5 weeks of age). They reported an improvement of 3.6 and 4.1 % in average daily feed intake and weight gain, respectively, due to fibre inclusion.

2.15.1 Type of fibre

The impact of dietary fibre on performance and nutrient digestibility largely depends on the fibre source because of the differences that exists in terms of physical and chemical properties (Mateos et al., 2012; Guzman et al., 2015). Hedge et al. (1978) observed that the inclusion of wheat bran at 100 g/kg diet significantly improved growth and F/G of chicks at 28 d post-hatch. Jorgensen et al. (1996) found that the reduction in nutrient digestibility was more pronounced in broilers fed diets containing oat bran than those fed diets containing pea fibre or wheat bran. The NSP concentration in the cell walls was suggested to be responsible for these differences observed. Hetland et al. (2003) reported that the inclusion of oat hulls in a broiler diet (cold-pelleted) increased gizzard weight and improved starch digestibility when compared to the control diet. They also observed an increase in amylase activity and concentration of bile acid in the jejunum due to the inclusion of oat hulls in broiler diets. Gonzalez-Alvarado et al. (2007) observed an improvement in growth performance of chicks fed diets containing oat and soy hulls. Gonzalez-Alvarado et al. (2010)

stated that the inclusion of oat hulls in a rice-based diet improved body weight gain and F/G of broilers compared to the control diet which was low in fibre. They observed an increase in gizzard weight with oat hulls inclusion in the diet. Furthermore, Liu et al. (2011) investigated the effect of 60 and 120 g/kg chicory (*Cichorium intybus* L.) forage as source of fibre in a wheat-based diet in broilers. They reported that in early stage of life of broiler the inclusion of chicory at 60 g/kg significantly increased body weight gain and improved feed utilisation efficiency compared to the control diet (without chicory). However, Walugembe et al. (2014) observed a reduction in average daily gain, and no effect on ADFI and AME in broilers fed diets containing wheat bran and dried distiller's grains with solubles (DDGS) when compared to broilers fed a diet with low fibre content.

The effect of dietary fibre in pullet/layer chickens has also been investigated. Hetland et al. (2003) reported that the inclusion of wood shavings in layer diets increased the gizzard weight by 50 %. In their study starch digestibility also improved. Guzman et al. (2015) used cereal straws (wheat/barley), sunflower hulls and sugar beet pulp as fibre sources in pullet diets. They reported that fibre type had no effect on ADFI, energy efficiency and F/G. A slight difference was observed between sunflower hulls and sugar beet pulp in terms of ADG. They speculated that the difference was due to physico-chemical differences, specifically associated with high levels of pectin in sugar beet pulp compared to sunflower hulls, which could have quickened satiety attainment (leading to reduced feed intake and growth rate).

2.15.2 Inclusion rate

To improve growth performance of broiler chicks, Gonzalez-Alvarado et al. (2007) recommended a 1.5 % inclusion of crude fibre in rice-based diets. In their study, the inclusion of 3 % soy and oat hulls in starter diets led to a significant improvement in utilisation of nutrients and body weight gain. They also observed that 3 % inclusion of dietary fibre increased the relative weight of gizzard and caeca. Guzman et al. (2015) showed that birds fed diet with 2 % dietary fibre inclusion (cereal straw, sugar beet pulp and sunflower hulls) had a lower F/G compared to birds fed diet with 4 % dietary fibre inclusion.

2.16 Economics of production

The aim of broiler production is to make profit, (Holsheimer and Ruesink, 1993). Therefore, every strategy or technology employed should be adequately evaluated to attain bird performance and economic benefits. One of the aspects worth considering is the nutrient density particularly the calorie levels in the diet, i.e. establishing optimal level with respect to the feed form that gives better performance as well as maximisation of profit.

Pellet feeding is another potential strategy to achieve desired economic benefits. Generally, it is to some extent accepted that pelleting process can incorporate feedstuffs that are less-palatable and inexpensive (Shen et al., 1985; Abdollahi et al., 2018b).

Some studies have compared the cost benefits in terms of production cost and profit in those fed pellets *vs.* mash. In a broiler study, Jahan et al. (2006) reported that birds fed pelleted diet had a low total cost of production per kg liveweight when compared to those fed mash diet. They also reported a higher profit associated with pellet feeding compared to mash feeding. Corzo et al. (2011) observed a better feed cost efficiency for diets containing 64 % pellets than the diet with zero percent pellet in broilers during 0 to 42 post-hatch.

2.17 Conclusion

- Nutrient density plays an important role in poultry diets, it has a substantial effect on bird growth performance regardless of the feed form, consequently affecting profit.
- Comparing mash diet, pelleting improves bird growth performance which is advantaged by enhanced feed intake. However, the development and functionality of digestive tract is negatively affected by pellet feeding compared to mash feeding.
- Optimal fibre level in broiler birds improves enzyme secretion and functionality of gizzard and proventriculus.

CHAPTER 3

Influence of nutrient density and feed form on growth performance, nutrient digestibility and gastro intestinal tract development in broilers fed wheat-based diets

3.1 Introduction

Pelleting is one of the common feed processing technology used in poultry industry. The benefits of pelleting over mash diet in terms of broiler growth performance have been demonstrated in several studies (Engberg et al., 2002; Amerah et al., 2007b; Jafarnejad et al., 2011; Chewning et al., 2012; Abdollahi et al., 2018b). The observed growth rate associated with pelleting is largely attributed to factors such as; increased feed intake, reduced ingredient segregation, less time and energy spent during feeding, and reduced feed wastage (Behnke, 2001; Peisker, 2006; Amerah et al., 2007b; Bolton, 1960). The magnitude of this growth performance advantage of pelleting tend to be affected by physical quality of pellet and dietary nutrient density levels (Abdollahi et al., 2011).

Feed intake is generally considered as the main driving factor for nutrient intake and performance of birds (Abdelsamie et al., 1983; Abdollahi et al., 2018a). However, dietary nutrient density has a substantial effect on feed intake of birds (Wiseman and Lewis, 1998). It has been reported that nutrient density have a considerable effect on growth rate (Waldroup et al., 1976), carcass characteristics and economics of production in meat-type chickens (Jackson et al., 1982; Jones and Wiseman, 1985). Possible interaction between nutrient density and feed form has been investigated in some studies (Lemme et al., 2006; Brickett et al., 2007; Jafarnejad et al., 2011). In the recent study, Abdollahi et al. (2018b) investigated the interactive effect of nutrient density and feed form on growth performance of broilers fed maize-based diets for 21 d post-hatch. They reported that pelleting improved weight gain by 33.9 % (249 g/bird) in VL diets and 6.0 % (54 g/bird) in VH diets in comparison to mash diets. They suggested that magnitude of benefits with pelleting on broiler performance varied depending on dietary nutrient density, with responses to pelleting decreasing with increasing dietary nutrient density.

The insufficient mechanical stimulation of pelleted diets is suggested to be the cause of poor development and functionality of gizzard observed in birds (Engberg et al., 2002). Therefore, since formulation of low dense diets allows the incorporation of fibre-rich ingredients (e.g. wheat bran and palm kernel meal), the negative effect of pelleting on the gut development could be reduced as well as improving the economic aspects. Abdollahi et al. (2018b) suggested that offering pelleted diets to birds, led to lighter gizzards at all nutrient densities; however, the reduction in relative gizzard weight was more pronounced in diets with higher nutrient densities.

Only few studies have investigated possible interaction between dietary nutrient density and feed form on broiler performance (Lesson et al., 1999; Lecznieski et al., 2001; Lemme et al., 2006; Brickett et al., 2007) and nutrient digestibility (Abdollahi et al., 2018b). It was hypothesised that feed form and dietary nutrient density will interact on performance and nutrient utilisation responses in broilers fed wheat-based diet. To test this hypothesis, the present study was designed to investigate the impact of feed form and dietary nutrient density on performance, nutrient and energy utilisation, GIT development and carcass characteristics responses for broiler chickens at 35 d post-hatch.

3.2 Materials and methods

3.2.1 Diets

A completely randomised design was used in this study, with a 5 x 2 factorial arrangement of 10 treatments (with 6 replicates, 8 birds per replicate) including five dietary nutrient densities and two feed forms. The nutrient density levels were; very low, VL; low, L; medium, M; high, H; and very high, VH and the feed form were pellet and mash. Total of 10 diets (5 starter, and 5 finisher) were formulated with wheat and soybean meal being the main ingredients. Other ingredients that were included to achieve the variation in nutrient densities were; full fat soybean, wheat bran, palm kernel meal, canola meal, maize gluten meal, soya oil as well as synthetic amino acids (lysine, methionine and threonine). To determine ileal nutrient digestibility, the indigestible marker, titanium dioxide (TiO2, Merck KGaA, Darmstadt, Germany) was added at the rate of 5.0 g/kg in finisher diets. Also, to lessen the effect of formulation of diets on pellet quality, a pellet binder (Mastercube; Kiotechagil, Reading, UK) was added at the rate of 2.0 g/kg in both feed form (mash and pellet) for starter and finisher diets. The ingredients were sourced from the commercial suppliers. The formulated diets varied in AMEn by 0.42 MJ/kg, lysine by 0.40 g/kg, methionine +

cysteine by 0.30 g/kg, and threonine by 0.27 g/kg. The formulation of medium nutrient densities was in accordance with Ross 308 nutrient recommendations (Ross, 2014). Tables 3.1 and 3.2 show the established variation pattern in basal diets of starter and finisher, respectively. The diets were similar in terms of ratios; protein to AME (18.3 g/MJ), lysine to AME (1.15 g/MJ), methionine + cysteine to lysine (0.75 g/g), and threonine to lysine (0.67 g/g), and finisher diet; protein to AME (15.8 g/MJ), lysine to AME (0.944 g/MJ), methionine + cysteine to lysine (0.776 g/g) and threonine to lysine (0.678g/g). The formulated diets were divided into two equal batches; steam-pelleted (conditioned at 75 °C for 30 seconds) and mash diets. Pelleting was done in the pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-upon-Hull, UK) which has the capacity to manufacture 180 kg of feed/h and is fitted with a die ring, with specifications of 3.0 mm-holes and 35 mm-depth. The pellet lengths of starter (3.0 mm) and finisher (6.0 mm) were achieved by adjusting the distance between the pellet die and knives, without any change in number of knives, pellet die, die rotation speed, and feeder rate. Representative samples of steam-pelleted diets were collected to determine pellet durability index (PDI).

3.2.2 Birds and housing

The experiment was conducted at Massey University poultry unit farm. The procedures employed were in accordance with the guidelines of Massey University Animal Ethics Committee. Four hundred and eighty-day-old male broilers (Ross 308) sourced from a commercial hatchery were weighed individually (to have similar average bird weight per cage) and distributed to 60 cages in electrically heated battery brooders. The birds were reared in 2 phases; starter (1-21 d) and finisher (22-35 d). During the first 12 days (d) post-hatch, each cage had 8 birds with the stocking density of 530 cm^2 per bird. While, for the remaining period (13-35 d) 120 cages were used, 4 birds per cage and the space allocation per bird was 640 cm^2. This was done to accommodate the increase in bird size, at the same time maintaining the respective replicates. Throughout the experimental period, birds were kept in an environmentally controlled poultry house supplying fluorescent illumination, 20 hours per day. On day 1, the temperature was at 31 °C and was then gradually reduced to 20 °C by d 35 of bird life. The nipple drinkers and feed troughs were provided in the cages. Fresh and clean water were readily available, and feed was provided ad libitum throughout the experiment period.

Table 3.1
Composition, calculated analysis and analysed values (g/kg as fed) of broiler starter diets (1-21 d)

	Nutrient density[a]				
Item	VL	L	M[b]	H	VH
Wheat	603.00	579.65	556.30	532.95	509.60
Soybean meal, 480 g/kg	219.20	218.08	216.95	215.83	214.70
Full fat soybean	5.00	30.00	55.00	80.00	105.00
Wheat bran	30.00	24.00	18.00	12.00	6.00
Palm kernel meal	30.00	24.00	18.00	12.00	6.00
Canola meal	40.00	32.00	24.00	16.00	8.00
Maize gluten meal	10.00	20.00	30.00	40.00	50.00
Soybean oil	14.70	23.93	33.15	42.38	51.60
Dicalcium phosphate	17.60	17.83	18.05	18.28	18.50
Limestone	11.50	11.18	10.85	10.53	10.20
L-Lysine HCl	4.80	4.98	5.15	5.33	5.50
DL-Methionine	3.70	3.80	3.90	4.00	4.10
L-Threonine	1.90	1.95	2.00	2.05	2.10
Sodium chloride	2.40	2.45	2.50	2.55	2.60
Sodium bicarbonate	2.20	2.18	2.15	2.13	2.10
Trace mineral premix[c]	1.00	1.00	1.00	1.00	1.00
Vitamin premix[c]	1.00	1.00	1.00	1.00	1.00
Pellet binder[d]	2.00	2.00	2.00	2.00	2.00
Xylanase	0.10	0.10	0.10	0.10	0.10
Calculated analysis					
Apparent metabolisable energy (AME), MJ/kg	11.71	12.13	12.55	12.97	13.39
Crude protein	215.00	222.50	230.00	237.50	245.00
Calcium	9.60	9.60	9.60	9.60	9.60
Non-phytate phosphorus	4.80	4.80	4.80	4.80	4.80
Sodium	2.00	2.00	2.00	2.00	2.00
Chloride	2.00	2.00	2.00	2.00	2.00
Potassium	8.20	8.30	8.40	8.50	8.60
Total lysine	13.44	13.92	14.40	14.88	15.36
Total methionine + cysteine	10.08	10.44	10.8	11.16	11.52
Total threonine	9.01	9.33	9.65	9.97	10.29
Ratio					
Crude protein to AME (g/MJ)	18.3	18.3	18.3	18.3	18.3
Lysine to AME (g/MJ)	1.15	1.15	1.15	1.15	1.15
Methionine + cysteine to lysine (g/g)	0.75	0.75	0.75	0.75	0.75
Threonine to lysine (g/g)	0.67	0.67	0.67	0.67	0.67
Analysed values					
Dry matter	908	917	931	933	934
Crude protein (Nitrogen × 6.25)	231.3	243.8	250.0	256.3	262.5
Starch	386	371	352	347	324
Fat	40.0	55.0	71.0	89.0	95.0
Calcium	10.3	11.0	10.0	10.2	9.7
Total phosphorus	7.9	8.7	8.2	8.2	8.0
PDI (%)[e]	86	82	81	72	65

[a] VL, very low, L, low; M, medium; H, high; VH, very high.

[b] Diet M was formulated to meet the Ross 308 strain recommendations for major nutrients (Ross, 2014).

[c] Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 12,000 IU; vitamin D3 (cholcalciferol), 4,000 IU; vitamin E (DL-α-tocopherol), 80 IU; biotin, 0.25 mg; pantothenic acid (D-Ca pantothenate), 15 mg; vitamin B12

(cyanocobalamin), 0.02 mg; folic acid, 3.0 mg; vitamin K3 (menadione nicotinamide bisulphite), 4.0 mg; niacin (nicotinic acid), 60 mg; pyridoxine (pyridoxine. HCl), 10 mg; riboflavin, 9.0 mg; thiamine (thiamine- mononitrate), 3.0 mg; antioxidant (ethoxyquin), 100 mg; choline (choline chloride 60%), 360 mg; Co (cobalt sulfate), 0.15 mg; Cu (copper sulfate), 6.0 mg; organic Cu (B-TRAXIM Cu), 3.0 mg; Fe (iron sulfate), 36 mg; I (calcium iodate), 0.93 mg; Mn (manganese oxide), 60 mg; Mo (sodium molybdate), 0.15 mg; Se (sodium selenite), 0.26 mg; organic Se (enriched yeast), 0.14 mg; Zn (zinc sulfate), 48 mg; organic Zn (B-TRAXIM Zn), 24 mg.

[d] Mastercube; Kiotechagil, Reading, UK.

[e] Pellet durability index of the pelleted diets (each value represents the mean of five replicate samples).

3.2.3 Pellet durability

Pellet durability index (PDI) of pelleted diets (starter and finisher) was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) in accordance to the method described by Abdollahi et al. (2010). Pellet samples weighing 100 g with no fines were rapidly circulated pneumatically around a perforated test chamber for thirty seconds. During the test cycle, fines were continuous removed through a 2.0 mm sieve. At the end of the test cycle, pellets were removed and weighed manually. The PDI was calculated as the relative proportion (by weight) of the retained pellets on 2.0 mm sieve to the whole pellets at the start.

3.2.4 Performance data and uniformity

Mortality was recorded daily throughout the experimental period. Feed intake and body weight of birds from each pen were recorded at weekly interval till the end of the trial. Feed per unit gain was corrected for body weight of any dead bird. Uniformity was determined based on the percentage of birds that were in the range of plus/minus 15 % from the mean average weight in each replicate, as described by Brickett et al. (2007).

3.2.5 Determination of metabolisable energy

Feed intake, and excreta output were collected from each cage over four consecutive days, d 18 to 21 post-hatch. The daily collected excreta from each pen were pooled, mixed in a blender and sub-sampled to determine dry matter (DM), N and gross energy (GE). Pending analysis, each sub-sample was freeze-dried (Model 0610, Cuddon Engineering, Blenheim, New Zealand), then ground to facilitate the passing of the sample through a 0.5mm sieve and stored in airtight plastic containers at 4 °C.

Table 3.2
Composition, calculated analysis and analysed values (g/kg as fed) of broiler finisher diets (21-35 d)

	Nutrient density[a]				
Item	VL	L	M[b]	H	VH
Wheat	713.10	681.93	650.75	619.58	588.40
Soybean meal, 480 g/kg	171.50	159.85	148.20	136.55	124.90
Full fat soybean	20.00	45.00	70.00	95.00	120.00
Wheat bran	10.00	8.00	6.00	4.00	2.00
Palm kernel meal	10.00	8.00	6.00	4.00	2.00
Canola meal	10.00	8.00	6.00	4.00	2.00
Maize gluten meal	10.00	22.50	35.00	47.50	6.00
Soybean oil	7.80	19.00	30.20	41.40	52.60
Dicalcium phosphate	15.40	15.48	15.55	15.63	15.70
Limestone	10.10	9.78	9.45	9.13	8.80
L-Lysine HCl	4.20	4.48	4.75	5.03	5.30
DL-Methionine	3.20	3.23	3.25	3.28	3.30
L-Threonine	1.60	1.65	1.70	1.75	1.80
Sodium chloride	2.30	2.33	2.35	2.38	2.40
Sodium bicarbonate	1.80	1.80	1.80	1.80	1.80
Trace mineral premix[c]	1.00	1.00	1.00	1.00	1.00
Vitamin premix[c]	1.00	1.00	1.00	1.00	1.00
Pellet binder[d]	2.00	2.00	2.00	2.00	2.00
Xylanase	0.10	0.10	0.10	0.10	0.10
Titanium dioxide (TiO2)	5.0	5.0	5.0	5.0	5.0
Calculated analysis					
Apparent metabolisable energy (AME), MJ/kg	12.13	12.55	12.97	13.39	13.81
Crude protein	191.80	198.40	205.00	211.60	218.20
Calcium	8.30	8.30	8.30	8.30	8.30
Non-phytate phosphorus	4.20	4.20	4.20	4.20	4.20
Sodium	1.90	1.90	1.90	1.90	1.90
Chloride	1.90	1.90	1.90	1.90	1.90
Potassium	7.10	7.10	7.20	7.20	7.20
Total lysine	11.46	11.85	12.25	12.65	13.04
Total methionine + cysteine	8.89	9.19	9.50	9.81	10.11
Total threonine	7.76	8.03	8.30	8.57	8.84
Ratio					
Crude protein to AME (g/MJ)	15.8	15.8	15.8	15.8	15.8
Lysine to AME (g/MJ)	0.944	0.944	0.944	0.944	0.944
Methionine + cysteine to lysine (g/g)	0.776	0.776	0.776	0.776	0.776
Threonine to lysine (g/g)	0.678	0.678	0.678	0.678	0.678
Analysed values					
Dry matter	903	912	939	932	937
Crude protein (Nitrogen × 6.25)	206.3	212.5	218.8	225.0	237.5
Starch	436	432	420	402	383
Fat	35.0	53.0	64.0	85.0	96.0
Calcium	10.7	9.0	9.1	8.7	7.8
Total phosphorus	8.9	7.4	7.6	7.5	7.3
PDI (%)[e]	96	96	89	80	91

[a] VL, very low, L, low; M, medium; H, high; VH, very high.

[b] Diet M was formulated to meet the Ross 308 strain recommendations for major nutrients (Ross, 2014).

[c] Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 12,000 IU; vitamin D3 (cholcalciferol), 4,000 IU; vitamin E (DL-α-tocopherol), 80 IU; biotin, 0.25 mg; pantothenic acid (D-Ca pantothenate), 15 mg; vitamin B12 (cyanocobalamin), 0.02 mg; folic acid, 3.0 mg; vitamin K3 (menadione nicotinamide bisulphite), 4.0 mg; niacin (nicotinic acid), 60 mg; pyridoxine (pyridoxine. HCl), 10 mg; riboflavin, 9.0 mg; thiamine (thiamine- mononitrate), 3.0 mg; antioxidant (ethoxyquin), 100 mg; choline (choline chloride 60%), 360 mg; Co (cobalt sulfate), 0.15 mg; Cu (copper sulfate), 6.0 mg; organic Cu (B-TRAXIM Cu), 3.0 mg; Fe (iron sulfate), 36 mg; I (calcium iodate), 0.93 mg; Mn (manganese oxide), 60 mg; Mo (sodium molybdate), 0.15 mg; Se (sodium selenite), 0.26 mg; organic Se (enriched yeast), 0.14 mg; Zn (zinc sulfate), 48 mg; organic Zn (B-TRAXIM Zn), 24 mg.

[d] Mastercube; Kiotechagil, Reading, UK.

[e] Pellet durability index of the pelleted diets (each value represents the mean of five replicate samples).

3.2.6 Determination of the coefficient of apparent ileal digestibility and gizzard pH

On day 35, three birds from each cage were euthanised by intravenous injection (1 mL per 2 kg live weight) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand). In accordance to a procedure described by Ravindran et al. (2005), ileal digesta were collected from the lower half of the ileum (after dividing ileum into two halves). Distilled water was used to gently flush the content of lower ileum into plastic containers. The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point of 40 mm proximal to the ileo-caecal junction. The collected ileal digesta from birds of respective cages were pooled, freeze-dried, ground to pass through a 0.5 mm sieve and then stored in airtight containers at 4 °C pending laboratory analysis. The ileal digesta samples and diets were analysed for DM, Ca, P, fat, N, GE, starch and titanium at the Nutrition Laboratory of the Institute of food Science and Technology, at Massey University -Manawatu Campus.

In addition, using the procedure described by Singh et al. (2014), the gizzard pH was measured from two of the birds (euthanised for ileal collection) per replicate. For each bird, the gizzard pH was measured at three different regions; proximal, middle and distal by inserting a calibrated digital pH meter (Model IQ120, ISFET pH Meter, Shindengen, Japan). The mean of the 3 readings was taken as pH of gizzard.

3.2.7 Upper digestive tract measurement and carcass characteristics.

On d 36, after six hours of fasting, two birds from each replicate (cage) with body weight closest to the mean weight of the cage were weighed and killed by cervical dislocation. The proventriculus and gizzard were then excised and emptied of the digesta content, also the adherent fat and mesentery were removed. The empty proventriculus and gizzards were rinsed, dried and weighed.

Carcass assessment was done on the same two birds (that supplied proventriculus and gizzard). The abdominal fat was manually removed and weighed. After evisceration, the empty carcass (without viscera, head, neck and feet) was weighed. The legs and breast muscle of each bird with respective to the replicate was weighed. The data were expressed as absolute weight (g) and as proportion of liveweight (g/kg body weight).

3.2.8 Chemical analysis

Dry matter of diets, excreta and ileal digesta was determined using standard procedures (Methods 930.15 and 925.10; AOAC, 2005). Ash was determined by standard procedures (method 942.05; AOAC, 2005) using a muffle furnace at 550 ºC for 16 hours. Ca and P were determined by colorimetric methods after ashing the samples at 550 ºC and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005). Adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) was used to determine gross energy, standardised with benzoic acid. The samples were tested for titanium on a UV spectrometer in accordance with the method described by Short et al. (1996).

The determination of N was done by combustion (Method 968.06; AOAC, 2005) using a carbon nanosphere-200 carbon, N and sulphur auto analyser (LECO Corporation, St. Joseph, MI). The content of starch was measured using the test method (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. For the determination of fat, the Soxhlet extraction method (Method 991.36; AOAC, 2005) was used.

3.2.9 Calculations

The apparent metabolisable energy was calculated using the following formula, with the correction for the differences in dry matter:

AME (MJ/kg diet) = $[(\text{FI x GE}_{\text{diet}}) - (\text{Excreta output x GE}_{\text{excreta}})] / \text{FI}$

The determination of N-corrected AME was by correcting for zero nitrogen retention by simple multiplication with 36.54 kJ/g N retained in the body, according to the description given by Hill and Anderson (1958).

The following formula was used to calculate the coefficient of apparent ileal digestibility (CAID):

CAID of diet nutrient = $[(\text{Nutrient/Ti})_{\text{diet}} - (\text{Nutrient/Ti})_{\text{ileal}}] / (\text{Nutrient/Ti})_{\text{diet}}$

Where (Nutrient/Ti)diet = ratio of nutrient to Ti in the diet, and

(Nutrient/Ti)ileal = ratio of nutrient to Ti in the ileal digesta.

3.2.10 Statistical analysis

To determine the main effect (feed form and nutrient density) data were analysed by two-way ANOVA, while General Linear Models (GLM) procedure of the SAS Institute Inc. (version 9.4; 2015) was used to determine their interaction. The differences were considered statistically significant at $P < 0.05$, and significant differences between means were separated by Least Significant Difference tests.

3.3 Results

3.3.1 Pellet durability index

The values of pellet durability index (PDI) were different at each level of dietary nutrient. At the nutrient density of VL, L, M, H and VH, the average PDI were, 86, 82, 81, 72 and 65 for starter diet, and 96, 96, 89, 80 and 91 for finisher diet, respectively, (Tables 3.1 and 3.2).

3.3.2 Growth performance

Starter period (d 1-21). Mortality of 2.5 % was recorded during this period and was not influenced ($P > 0.05$) by dietary treatments. At each nutrient density level, weight gain of birds fed pelleted-diets was higher than those fed mash diets. The magnitude of increase in weight gain associated with pelleting over mash feeding reduced gradually with increase in nutrient density, resulting in a significant ($P < 0.01$; Table 3.3) nutrient density x feed form interaction for weight gain. Birds fed pellet diets had similar weight gain, however, in mash diets, weight gain improved as the nutrient density increased, with birds fed M diets having higher weight gain than those fed VL diet, but lower than those fed H and VH diets.

Birds fed pelleted-diets consumed more ($P < 0.001$) feed than those fed mash diet. Increase in nutrient density had a significant ($P < 0.001$) influence on feed intake, with birds fed VL diets having the highest feed intake compared to other groups. However, birds fed L, M, H and VH had similar feed intake.

A significant ($P < 0.001$) interaction between nutrient density and feed form was observed for F/G. Feed per unit gain was not influenced by pelleting in VL, L and M diets. However, in H and VH diets the F/G deteriorated due to pelleting when compared to mash diets.

Finisher period (d 21-35). During this period mortality was 1.9 %. There was a tendency for mortality to increase ($P = 0.068$) as the nutrient density levels increased, also pellet fed birds had a slightly higher ($P = 0.084$) mortality than those fed mash diets. The interaction between nutrient density and feed form was significant ($P < 0.05$; Table 3.4) for weight gain. Pelleting improved weight again at each nutrient density, and the advantages of pelleting over mash diet reduced as the nutrient density increased. In mash diets, birds fed H diets had higher weight gain than those fed VL diets, and similar to those fed L, M and VH diets. In pellet diets, birds fed VH diet had lower weight gain that those fed VL and L diets, but similar to those fed M and H diets.

Table 3.3
Influence of feed form and nutrient density on the weight gain (g/bird), feed intake (g/bird), feed per unit gain (F/G, g feed/g gain), mortality (%) in broiler starters (d 1 - 21)[a]

Item		Growth performance			
Nutrient density	Feed form	Weight gain	Feed intake	F/G	Mortality
VL	Mash	799d	1057	1.331a	0.0
	Pellet	1030a	1354	1.318a	4.2
L	Mash	811cd	1029	1.281b	4.2
	Pellet	1006a	1283	1.279b	4.2
M	Mash	839c	1018	1.246cd	2.1
	Pellet	1015a	1281	1.262bc	2.1
H	Mash	881b	1033	1.199e	0.0
	Pellet	1033a	1267	1.234d	4.2
VH	Mash	909b	1033	1.149f	4.2
	Pellet	1039a	1247	1.200e	0.0
Pooled SEM		13.5	15.8	0.0076	2.73
Main effects					
Nutrient density					
VL		915	1205a	1.324	2.1
L		909	1156b	1.280	4.2
M		927	1149b	1.254	2.1
H		957	1150b	1.217	2.1
VH		974	1140b	1.175	2.1
Feed form					
	Mash	848	1034b	1.241	2.1
	Pellet	1025	1286a	1.259	2.9
Probabilities, P ≤					
Nutrient density		0.001	0.001	0.001	0.919
Feed form		0.001	0.001	0.001	0.632
Nutrient density x Feed form		0.01	0.120	0.001	0.522

Very low = VL, Low = L; Medium = M; High = H; Very high = VH.

Means not sharing same letter (a-f) are significantly different ($P < 0.05$).

[a] Each value is a representation of mean of 6 replicates (8 birds per replicate).

A significant ($P < 0.01$) interaction between nutrient density and feed form was observed for feed intake. Birds fed mash diets had low feed intake than those fed pellet diets at each nutrient density level, and the magnitude of increase in feed intake associated with pelleting reduced gradually with increase in nutrient density.

Table 3.4

Influence of feed form and nutrient density on the weight gain (g/bird), feed intake (g/bird), feed per unit gain (F/G, g feed/g gain), mortality (%) in broiler finisher (d 21 - 35)[a]

Item		Growth performance			
Nutrient density	Feed form	Weight gain	Feed intake	F/G	Mortality
VL	Mash	1236d	2056d	1.664	0.0
	Pellet	1541a	2550a	1.715	0.0
L	Mash	1295cd	2045d	1.616	0.0
	Pellet	1588a	2526a	1.617	0.0
M	Mash	1303cd	2015de	1.574	0.0
	Pellet	1528ab	2348b	1.620	6.6
H	Mash	1325c	1999de	1.544	2.1
	Pellet	1522ab	2308b	1.546	2.4
VH	Mash	1312cd	1944e	1.545	2.8
	Pellet	1449b	2211c	1.548	6.3
Pooled SEM		30.4	33.9	0.0225	1.85
Main effects					
Nutrient density					
VL		1389	2303	1.689a	0.0
L		1442	2285	1.616b	0.0
M		1415	2181	1.597b	3.3
H		1423	2154	1.545c	2.2
VH		1380	2077	1.547c	4.5
Feed form					
	Mash	1294	2012	1.589	1.0
	Pellet	1526	2388	1.609	3.0
Probabilities, P ≤					
Nutrient density		0.259	0.001	0.001	0.068
Feed form		0.001	0.001	0.157	0.084
Nutrient density x Feed form		0.046	0.01	0.636	0.310

Very low = VL, Low = L; Medium = M; High = H; Very high = VH.

Means not sharing same letter (a-e) are significantly different ($P < 0.05$).

[a] Each value is a representation of mean of 6 replicates (8 birds per replicate).

Neither the main effect of feed form nor the interaction between nutrient density and feed form was significant ($P > 0.05$) for F/G. Nutrient density significantly ($P < 0.001$) affected the F/G, with birds fed VL diet having the highest and those fed the H and VH diets had lowest F/G values.

Whole trial period (d 1-35). The effects of dietary treatments on weight gain, feed intake, F/G, mortality and uniformity are shown in Table 3.5. Mortality of 4.4 % was recorded during the entire experiment period, but not affected ($P > 0.05$) by dietary treatments. Birds fed pelleted diets had higher ($P < 0.01$) uniformity percentage than those fed mash diets.

Significant (P < 0.001) interactions between nutrient density and feed form were observed for weight gain during whole trial period. While birds fed pelleted diets outperformed those fed mash diets at each nutrient density level, the pellet-associated benefits on weight gain were more pronounced at the lowest nutrient density.

Table 3.5
Influence of feed form and nutrient density on the weight gain (g/bird), feed intake (g/bird), feed per unit gain (F/G, g feed/g gain), mortality (%), uniformity in broiler (d 1 - 35)[a]

Item		Growth performance				
Nutrient density	Feed form	Weight gain	Feed intake	F/G	Mortality	Uniformity
VL	Mash	2036e	3113d	1.532	0.0	89.7
	Pellet	2571ab	3904a	1.548	4.2	96.0
L	Mash	2106de	3073de	1.481	4.2	95.2
	Pellet	2595a	3809a	1.477	4.2	91.2
M	Mash	2141cd	3033de	1.440	2.1	79.7
	Pellet	2543ab	3629b	1.466	8.3	92.0
H	Mash	2206cd	3031de	1.398	2.1	80.7
	Pellet	2555ab	3575b	1.412	6.3	100.0
VH	Mash	2221c	2977e	1.367	6.3	83.3
	Pellet	2488b	3457c	1.395	6.3	91.7
Pooled SEM		36.6	41.5	0.0105	3.64	5.34
Main effects						
Nutrient density						
VL		2303	3509	1.540a	2.1	92.8
L		2350	3441	1.479b	4.2	93.2
M		2342	3331	1.453c	5.2	85.8
H		2380	3303	1.405d	4.2	90.3
VH		2354	3217	1.381e	6.3	87.5
Feed form						
	Mash	2142	3045	1.444b	2.9	85.7b
	Pellet	2550	3675	1.459a	5.8	94.2a
Probabilities. P <						
Nutrient density		0.339	0.001	0.001	0.836	0.574
Feed form		0.001	0.001	0.022	0.212	0.015
Nutrient density x Feed form		0.01	0.01	0.592	0.880	0.288

Very low = VL, Low = L; Medium = M; High = H; Very high = VH.
Means not sharing same letter (a-e) are significantly different (P < 0.05).
[a] Each value is a representation of mean of 6 replicates (8 birds per replicate).

A significant (P < 0.01) interaction between nutrient density and feed form was observed for feed intake. At each nutrient density level, feed intake was higher in birds fed pelleted-diets compared to those fed mash diets, with the advantages of pelleting reducing as the nutrient density increased. In mash diets, feed consumption decreased with the increase in nutrient density, with birds fed VL diet having higher feed intake than those fed VH diet, but similar to those fed L, M and H diets. Similarly, in pellet diets, increasing nutrient density decreased the amount of feed consumed. However, birds fed VL and L diets had the highest and those fed VH diet had the lowest feed intake.

Pelleting increased ($P < 0.05$) F/G when compared to mash diet. Increasing nutrient density decreased ($P < 0.001$) the F/G. No significant ($P > 0.05$) interaction between feed form and nutrient density was observed for the F/G.

3.3.3 Nutrient and energy utilisation

A significant ($P < 0.05$; Table 3.6) nutrient density x feed form interaction was observed for the CAID of dry matter (DM). Pelleting did not affect CAID of DM in VL, L and H diets, but reduced that of M and VH diets. In mash diet, increasing nutrient density increased the CAID of DM, with VL diet having the lowest and VH having the highest CAID of DM. In pellet diets, CAID of DM was also influenced by nutrient density, with M diets having higher CAID of DM than VL diets, but lower than that of L, H and VH.

No significant ($P > 0.05$) feed form x nutrient density interaction was observed for CAID of N. Pelleting lowered ($P < 0.05$) CAID of N compared to mash diets. Nutrient density affected ($P < 0.001$) CAID of N, with L and M diets having a lower CAID of N than that of H and VH, but higher than that of VL diets.

Comparing mash diets, pelleting lowered ($P < 0.001$) the CAID of starch. There was a tendency ($P = 0.091$) for nutrient density to influence CAID of starch, with VL diet having higher CAID of starch compared to M and VH diets. No significant ($P > 0.05$) nutrient density x feed form interaction was observed for CAID of starch.

Pelleting lowered ($P < 0.001$) CAID of fat compared to mash diet. The main effect of nutrient density was significant ($P < 0.001$) on CAID of fat, with H and VH diets having the higher CAID of fat compared to VL and L diets.

Table 3.6

Influence of feed form and nutrient density on the coefficient of apparent ileal digestibility (CAID)[a] of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca), phosphorous (P), gross energy (GE)[a], GE metabolisability[b] and nitrogen-corrected apparent metabolisable energy (AMEn, MJ/kg DM)[b] in broilers (1-35 d).

Item		CAID						Energy utilisation		
Nutrient density	Feed form	DM	N	Starch	Fat	Ca	P	CAID of GE	GE metabolisability	AMEn
VL	Mash	0.546e	0.750	0.954	0.790	0.153	0.282	0.590e	0.677	12.47
	Pellet	0.539e	0.745	0.928	0.754	0.154	0.248	0.576e	0.667	12.29
L	Mash	0.648c	0.817	0.950	0.889	0.273	0.368	0.683cd	0.691	12.99
	Pellet	0.658bc	0.796	0.906	0.858	0.333	0.421	0.691c	0.675	12.68
M	Mash	0.655c	0.824	0.941	0.912	0.265	0.388	0.707bc	0.706	13.58
	Pellet	0.594d	0.775	0.878	0.870	0.349	0.433	0.643d	0.681	13.08
H	Mash	0.683abc	0.838	0.937	0.911	0.302	0.477	0.720abc	0.705	13.85
	Pellet	0.702ab	0.832	0.906	0.909	0.366	0.537	0.739ab	0.694	13.63
VH	Mash	0.725a	0.854	0.919	0.923	0.346	0.496	0.757a	0.716	14.24
	Pellet	0.666bc	0.828	0.904	0.889	0.202	0.435	0.713bc	0.699	13.91
Pooled SEM		0.0160	0.0111	0.0125	0.0111	0.0421	0.0264	0.0146	0.0038	0.073
Main effects										
Nutrient density										
VL		0.542	0.748c	0.941	0.772c	0.154b	0.265c	0.583	0.672d	12.38e
L		0.653	0.807b	0.928	0.874b	0.303a	0.394b	0.687	0.683c	12.83d
M		0.624	0.800b	0.909	0.891ab	0.307a	0.411b	0.675	0.694b	13.33c
H		0.693	0.835a	0.922	0.910a	0.334a	0.507a	0.729	0.700b	13.74b
VH		0.696	0.841a	0.912	0.906a	0.274a	0.466a	0.735	0.707a	14.08a
Feed form										
	Mash	0.651	0.817a	0.940a	0.885a	0.268	0.402	0.691	0.699a	13.43a
	Pellet	0.632	0.795b	0.904b	0.856b	0.281	0.415	0.672	0.683b	13.12b
Probabilities, P ≤										
Nutrient density		0.001	0.001	0.091	0.001	0.001	0.001	0.001	0.001	0.001
Feed form		0.060	0.004	0.001	0.001	0.632	0.459	0.042	0.001	0.001
Nutrient density x Feed form		0.034	0.270	0.392	0.431	0.057	0.080	0.034	0.272	0.232

Very low=VL, Low=L; Medium=M; High=H; Very high=VH.

Means not sharing same letter (a-e) are significantly different ($P < 0.05$).

GE metabolisability = AMEn / GE content of the diet.

[a] Each value represents the mean of six replicates (8 birds per replicate), measured on d 35.

[b] Each value represents the mean of six replicates (8 birds per replicate), measured from d 17 to 21 post-hatch.

Increasing nutrient density significantly ($P < 0.001$) affected CAID of Ca, VL diet had the lowest CAID of Ca among all, while L, M, H and VH diets had similar CAID of Ca. The effect of feed form on CAID of Ca was not significant ($P > 0.05$). There was a tendency ($P = 0.057$) for interaction between nutrient density and feed form for CAID of Ca.

Nutrient density had a significant ($P < 0.001$) influence on CAID of P, the L and M diets had higher CIAD of P than that of VL diet, but lower than that of H and VH diets. There was no effect ($P > 0.05$) of feed form on CAID of P. A tendency ($P = 0.080$) was observed for nutrient density x feed form interaction for CAID of P

A significant ($P < 0.05$) interaction between nutrient density and feed form was observed for CAID of GE. Pelleting had no effect in VL, L, H diets but it reduced CAID of GE in M and VH diets compared to mash diets.

Nutrient density influenced GE metabolisability and AMEn in a similar direction ($P < 0.001$), with gradual increases with increasing nutrient densities. Feeding pelleted diets, regardless of nutrient density, reduced ($P < 0.001$) GE metabolisability and AMEn. No interaction ($P > 0.05$) was observed between nutrient density and feed form for the energy utilisation parameters assessed.

3.3.4 Upper tract development and gizzard pH

Absolute empty weight of proventriculus (g) was not influenced ($P > 0.05$) by dietary treatments. Birds fed mash diets had higher ($P < 0.001$; Table 3.7) absolute empty weight of gizzard than those fed pellet diets. Neither main effect of nutrient density nor the interaction between nutrient density and feed form was significant ($P > 0.05$) for the absolute weight of gizzard.

A significant ($P < 0.01$) nutrient density and feed form interaction was observed for relative (g/kg body weight) proventriculus weights. In VH diets, pelleting did not influence relative proventriculus weight, but in VL, L, M and H diets pellet feeding decreased the proventriculus weight. In mash diets, birds fed L nutrient density had higher relative weight of proventriculus than those fed H and VH diets, but similar to those fed VL and M diets. In pelleted diets, relative weight of proventriculus of birds fed VL diets was lower than those fed M diets, though similar to those fed L, H and VH diets.

Table 3.7

Influence of nutrient density and feed form on the absolute (g) and relative (g/kg body weight) weight of upper digestive tract and gizzard pH[a] of 36 d old broilers[b]

Item		Absolute weight		Relative weight (g/kg)		
Nutrient density	Feed form	Proventriculus	Gizzard	Proventriculus	Gizzard	Gizzard pH
VL	Mash	5.62	19.5	2.50ab	8.98a	2.75
	Pellet	5.09	14.1	1.92g	5.26c	3.74
L	Mash	5.71	19.6	2.61a	8.91a	2.76
	Pellet	5.59	14.3	2.05efg	5.28c	3.21
M	Mash	5.88	19.7	2.40abc	8.95a	2.71
	Pellet	5.60	14.2	2.17def	5.32c	3.58
H	Mash	5.56	19.2	2.29bcd	8.45a	3.12
	Pellet	5.27	14.4	1.97fg	5.28c	3.60
VH	Mash	5.20	18.4	2.20cde	7.80b	2.59
	Pellet	5.71	13.8	2.11defg	5.23c	3.59
Pooled SEM		0.212	0.43	0.081	0.214	0.162
Main effects						
Nutrient density						
VL		5.36	16.81	2.21	7.12	3.24
L		5.65	16.96	2.33	7.10	2.98
M		5.74	16.95	2.28	7.13	3.14
H		5.41	16.78	2.13	6.87	3.36
VH		5.46	16.09	2.16	6.51	3.09
Feed form						
	Mash	5.59	19.26a	2.40	8.62	2.79b
	Pellet	5.45	14.18b	2.04	5.27	3.54a
Probabilities, P ≤						
Nutrient density		0.319	0.238	0.081	0.019	0.187
Feed form		0.297	0.001	0.001	0.001	0.001
Nutrient density x Feed form		0.154	0.767	0.014	0.044	0.231

VL, very low, L, low; M, medium; H, high; VH, very high.

Means in a column not sharing a common letter (a-g) are significantly different ($P < 0.05$).

[a] Each value represents the mean of six replicates (two gizzards per replicate, three pH readings per gizzard).

[b] Each value represents the mean of six replicates (two birds per replicate).

Table 3.8

Influence of nutrient density and feed form on relative (g/kg of body weight)[a] weight of carcass characteristics of broilers slaughtered at d 36 of age

Item		Relative weight (g/kg body weight)			
Nutrient density	Feed form	Carcass	Breast	Legs	Fat
VL	Mash	722	219	181	5.09
	Pellet	739	240	181	4.89
L	Mash	724	208	182	5.00
	Pellet	738	236	182	4.56
M	Mash	723	222	175	5.20
	Pellet	737	234	181	5.01
H	Mash	720	213	180	5.58
	Pellet	739	233	178	5.45
VH	Mash	730	222	175	5.18
	Pellet	740	232	180	4.96
Pooled SEM		2.8	4.8	2.7	0.386
Main effects					
Nutrient density					
VL		731	229	181	4.99
L		731	222	182	4.78
M		730	228	178	5.10
H		730	223	179	5.52
VH		735	227	178	5.07
Feed form					
	Mash	724b	217b	178	5.21
	Pellet	739a	235a	181	4.97
Probabilities, P ≤					
Nutrient density		0.319	0.508	0.410	0.426
Feed form		0.001	0.001	0.213	0.330
Nutrient density x Feed form		0.627	0.310	0.442	0.996

VL, very low, L, low; M, medium; H, high; VH, very high.

[a] Each value represents the mean of six replicates (eight birds per replicate).

A significant ($P < 0.01$) nutrient density x feed form interaction was observed for relative (g/kg body weight) gizzard weights. Feeding pelleted diets led to lighter gizzards at all nutrient densities, however, the reduction in relative gizzard weight by pelleting was smaller in the diet with VH nutrient density. Birds fed pellet diets had similar relative gizzard weight, while in mash diets, birds fed VH diet had the lowest relative gizzard weight compared to others.

Birds fed pelleted-diets had higher ($P < 0.001$) gizzard pH than those fed mash diet. Neither main effect of nutrient density nor interaction between nutrient density and feed form were significant ($P > 0.05$) for gizzard pH.

3.3.5 Carcass characteristics

The effects of dietary treatments on relative carcass yield are summarised in Table 3.8. Birds fed pellet diets had higher ($P < 0.001$) carcass and breast weights compared to those fed mash diets. Neither main effect of nutrient density nor the interaction between nutrient density and feed form were significant ($P > 0.05$) for relative weight of carcass.

3.4 Discussion

In the present study, during the starter period (d 1-21), birds fed steam-pelleted diets outperformed those fed mash diets at each nutrient density level, however, this advantage of pelleting declined with the increase in nutrient density. Likewise, this pattern was observed during the finisher (d 21- 35) and whole grow-out period (d 1-35). During the whole experimental period, pelleting improved weight gain by 19.0 % (2550 *vs.* 2142 g/bird), but this advantage was gradually reduced from 26.3 % (2571 *vs.* 2036 g/bird) in VL diets to 12.0 % (2488 *vs.* 2221) in VH diets. Allred et al. (1957) reported that the benefit of pelleting in terms of weight gain was greater in poults fed diets with low nutrient density compared to those fed diets with high nutrient density. Bayley et al. (1968) reported that when low (2400 kcal/kg) and high (2560 kcal/kg) energy dense mash maize-based diets were crumbled, weight gain of chicks improved by 19.7 % (58 g/bird) and 12.3 % (37 g/bird), respectively. Brickett et al. (2007) also observed the reduction in the advantages of pelleting over mash diets with the increase in nutrient density, from 2800 to 3100 kcal AME/kg. They reported that the feed intake diminished from 17.4 % in low nutrient dense (3112 *vs.* 2650 g/bird) to 13.4 % in high nutrient dense pelleted diets (2927 *vs.* 2582 g/bird), likewise, body weight improvement reduced from 17.7 % (289 g) to 10.8 % (188 g) in low and high density diets, respectively. In a recent study, Abdollahi et al. (2018b) found a significant interaction between nutrient density and feed form on weight gain of broiler starters fed maize-based diets. They reported a weight gain improvement of 17.1 % (143 g/bird) due to pellet feeding, which reduced progressively from 33.9 % (249 g/bird) in VL diets to 6.0 % (54 g/bird) in VH diets as the nutrient density levels increased. The beneficial effect of pelleting on growth performance in comparison to mash diets have been demonstrated in several studies (Engberg et al., 2002; Jahan et al., 2006; Amerah et al., 2007b; Abdollahi et al., 2013b, 2018b), and have been largely attributed to higher feed intake resulting from increased bulk density of pelleted rations facilitating prehension. High feed intake is generally associated with higher intake of energy and nutrients, resulting in improved growth response of pellet-fed birds (Abdollahi et al., 2013a, 2014).

During the starter period, birds fed pelleted diets consumed more ($P < 0.001$) feed than those fed mash diets. Also, regardless of feed form, increasing nutrient density significantly influenced feed intake, with birds fed VL diets having the highest feed intake, but no significant ($P > 0.05$) interaction of the main effects was observed. This lack of interaction between nutrient density and feed form for feed intake during the starter period was due to the fact that both feed forms (pellet and mash) had a similar pattern in response to increased nutrient density levels. However, interaction between nutrient density and feed form was significant for feed intake during finisher and whole trial period. During whole trial period, pelleted diets supported higher feed intake by 20.7% (3675 *vs.* 3045 g/bird), but this advantage was eroded from 24.4% in VL diets (3904 *vs.* 3113 g/bird) to 16.1% in VH diets (3457 *vs.* 2977 g/bird). An early study by Jensen and McGinnis (1952) evaluated the performance response of laying hens to mash and pelleted diets containing different inclusions of alfalfa. These researchers found that when the level of alfalfa increased from 100 to 150, 200 and 250 g/kg in mash diets, birds lost more weight, whereas pellet-fed hens gained substantial weight. These researchers also observed that hens fed pelleted diets consumed more feed than the corresponding mash diets with the feed intake differences being larger in high alfalfa (200 and 250 g/kg) diets. However, Jones and Wiseman (1985) reported lack of significant effect on feed intake in broiler chicks (1-24 d) as dietary energy levels increased from 2575, 3053 to 3530 kcal ME/kg with an isonitrogenous of 23 g/kg in mash wheat-based diets. These researchers indicated that AME intake increased with increase in dietary energy content, while protein intake remained the same because of the isonitrogenous diets. Brickett et al. (2007) also observed that increase in dietary energy content did not influence feed intake of broiler chicks fed barley-maize-wheat pelleted diets at 6 d post-hatch, regardless of feed form. However, Abdollahi et al. (2018b) observed a significant interaction between nutrient density and feed form for feed intake in broiler chicks fed maize-based starter diets.

In contrast to finisher and whole grow-out period, the effect of pelleting on F/G was inconsistent across the nutrient density levels during starter period, resulting in a significant feed form x nutrient density interaction. The F/G was not influenced by pelleting in VL, L and M diets, however, in H and VH diets pelleting deteriorated F/G by 2.9 % (1.234 *vs.* 1.199) and 4.4 % (1.200 *vs.* 1.149), respectively. In agreement, Abdollahi et al. (2018b) showed that the magnitude of F/G improvement associated with pelleting is dependent on nutrient density levels. They reported that pelleting improved F/G by 6.2 % in VL diets (1.373 *vs.* 1.463) while in VH diets it deteriorated F/G by 3 % (1.207 *vs.* 1.172). The overall deterioration of F/G due to pellet feeding observed in the present study agrees with Abdollahi et al. (2011), who found that pelleted diets deteriorated F/G compared to mash diets in broilers fed wheat-based diets for 21 d post-hatch. In the current study, increasing nutrient density levels improved F/G during each growth phase, regardless of the feed form. This finding is consistent with that of Jackson et al. (1982), and Holsheimer and Veerkamp (1992) who reported an improvement of F/G as the dietary energy increased.

In comparison to mash diets, pelleting reduced the digestibility of N by 2.7, fat by 3.3 and starch by 3.8 %. These results are in agreement with those of Abdollahi et al. (2013b) who reported that feeding a wheat-based pelleted diet to broilers reduced digestibility of these nutrients. In a recent study, Abdollahi et al. (2018b) reported that in birds fed maize-based diets, pelleting reduced the digestibility of N, starch and fat by 9.7, 2.7 and 4.4 %, respectively. The low digestibility of starch in wheat-based diets is mainly attributed to increased load of starch in the gut due to high feed intake (Svihus and Hetland, 2001).

Nutrient density effect on digestibility of minerals might depend on ingredient composition of the diet. This finding is difficult to explain, but there is the possibility that in the diets with VL nutrient density, mineral-lipid complex formation form intact fat is higher. Soybean oil inclusion increased from 7.8 to 52.6 g/kg in VL to VH finisher diets as dietary nutrient densities increased. However, Abdollahi et al. (2018b) suggested that the formation of mineral-lipid complex during pelleting process is responsible for variation observed in mineral digestibility with respect to changes in dietary fat levels. But in current study, neither the main effect of pelleting nor the interaction of nutrient density and pelleting was significant.

Pelleting reduced AMEn by 2.4 % (13.12 *vs.* 13.43 MJ/kg DM), and these results match those observed in earlier studies (Amerah et al., 2007b; Abdollahi et al., 2011, 2013b). Amerah et al. (2007b) found that pelleting reduced AMEn by 2.2 % (2820 *vs.* 2884 kcal/kg DM) in birds fed wheat-based diet at 21 d post-hatch. Further, as expected, increasing dietary nutrient density increased AMEn, gross energy (GE) metabolisability and CAID of GE. Similar findings were reported by Abdollahi et al. (2018b) who attributed this observation to increased amount of soybean oil added to the diets.

Increasing nutrient density had no significant effect on the absolute weight of proventriculus and gizzard, however, pelleting lowered absolute weight of gizzard by 26.4 % (14.18 *vs.* 19.26 g) and increased gizzard pH by 26.9 % (3.54 *vs.* 2.79). These results are in line with those of Engberg et al. (2002), who observed a significant reduction in gizzard weight, and an increase in gizzard pH associated with pellet feeding. The under-development of gizzard in pellet fed birds has also been observed in several other studies (Abdelsamie et al., 1983; Svihus et al., 2004; Amerah et al., 2007b). This observation is largely attributed to inadequate mechanical stimulation associated with pelleted diets, reduced retention time of feed in upper gut compartments, and insufficient stimulation of proventriculus for the secretion of hydrochloric acid (Engberg et al., 2002). In the present study, the effect of pelleting on relative proventriculus and gizzard weight was inconsistent across the nutrient density levels, leading to a significant feed form x nutrient density interaction. Feeding pelleted diets lowered the relative weight of proventriculus, except in birds fed VH diets where pelleting had no effect. Feeding pelleted diets led to lighter gizzards at all nutrient densities; however, the reduction in relative gizzard weight was less in diets with very high nutrient density. This highlights the role of structural component in gizzard development and the sensitivity of the gizzard to feed structure and its development. These results are in line with those of Abdollahi et al., 2018b. They found pelleting to have an inconsistent influence on relative gizzard weight across the different nutrient density levels in broilers fed maize-based diets.

Increasing nutrient density did not influence relative weight of abdominal fat pad. This outcome is contrary to that of Jones and Wiseman (1985) who found that abdominal fat was lower in birds fed a low energy diet than those fed a high energy diet. In their study, the diets were formulated to have 2576, 3054 and 3533 kcal AME/kg with of 230 and 200 g/kg protein for starter and finisher phases, respectively. Lemme et al. (2006) also observed a decrease in abdominal fat with the increase in protein level in broiler diets. While in the current study, CP and other nutrients were adjusted with respect to the changes in energy, and this could be the possible explanation for this difference observed. Pellet feeding improved the relative weight of carcass and breast by 2.1 % (15 g) and 8.3 % (18 g), respectively. This finding is consistent with that of Corzo et al. (2011) who reported that broilers fed pelleted maize-based diets had higher carcass and breast weight than those fed mash diets at d 42. Further, the lack of interaction of the main effects on carcass characteristics in the current study is in accordance with the findings of Brickett et al. (2007) who observed no significant interaction between nutrient density and feed form for relative abdominal fat and breast weight.

An overall mortality of 4.4 % was recorded in this study and was not related to the dietary treatments. Ascites, sudden death syndrome (SDS) and skeletal problems mortalities have been increasingly reported in broiler production due to fast-growth of broilers, specifically broilers that have been fed pelleted diets (Engberg et al., 2002). However, in the present study the influence of pelleting on mortality was not significant, which is in agreement with the results of Scott (2002) and Cerrate et al. (2009) who found that mortality was not affected by feed form. In present study, increasing nutrient density did not influence mortality during whole trial period. These results agree with the results of Skinner et al. (1992) and Brickett et al. (2007).

Effect of feed form was significant ($P < 0.01$) on bird uniformity, with pelleting having 10% higher uniformity compared to mash diets. This observation is consistent with the results of Brickett et al. (2007) who found that mash fed birds had poor uniformity.

Abdollahi et al. (2018b) found that increasing nutrient density impaired physical quality of pelleted diets. This observation supports the finding of the present study in which PDI of pelleted starter diets reduced as the nutrient density levels increased. The lubricative effect of fat reduces the compaction of feed in the die holes due to low frictional force generated in these die holes, resulting in poor pellet quality as dietary fat levels increase (Abdollahi et al., 2013a). Also, dietary fat tends to partially encapsulate feed particles, reducing steam penetration and gelatinization of starch, leading to poor development of adhesion forces (Lowe, 2005). On the other hand, contrary to the expectations high PDI was observed in VH finisher diet. This finding is difficult to explain.

In summary, the effect of nutrient density and feed form on broiler growth performance is not limited to starter period, but it persists throughout the growth period of 35 d. Pelleting-induced benefits in terms of weight gain and feed intake decreased with increase in dietary nutrient density. Manipulation of dietary nutrient density influenced digestibility of major nutrients, but it does not affect carcass characteristics. As expected, pelleting reduced the proventriculus and gizzard weight regardless of the nutrient density levels. Further, since the low dense diet is achieved through the incorporation of relatively inexpensive fibrous raw material, feeding of low dense pelleted-diets can help in reducing cost of feed thereby maximising profit.

3.5 Conclusions

The magnitude of increase in broiler growth performance associated with pelleting depend on dietary nutrient density level, pelleting advantages are greater at the lowest nutrient density level. Irrespective of nutrient density level, pelleting did not enhance digestibility of nutrients, however, high nutrient intake facilitated by high feed intake recompense this negative effect of pelleting on nutrients digestibility. The present study also highlights that dietary nutrient density must be considered if the full benefits associated with feeding pelleted diets are to be achieved. The findings of this study has demonstrated that the benefits of pelleting over mash can be enhanced by use of low dense diets in comparison to the current density levels used in pelleted diets.

REFERENCES

Abdelsamie, R. E., Ranaweera, K. N. and Nano, W. E. (1983) The influence of fibre content and physical texture of the diet on the performance of broilers in the tropics. Poultry Science 24: 383-390.

Abdollahi, M. R. and Ravindran, V. (2012) The quest for the perfect pellet: Issues and challenges. Proceedings of the New Zealand Poultry Industry Conference, Volume 11.

Abdollahi, M. R., Ravindran, V. and Svihus, B. (2013a) Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Animal Feed Science and Technology 179: 1-23

Abdollahi, M. R., Ravindran, V. and Svihus, B. (2013b) Influence of grain type and feed form on performance, apparent metabolisable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. Animal Feed Science and Technology 186: 193-203.

Abdollahi, M. R., Ravindran, V. and Svihus, B. (2014) Influence of feed form on growth performance, ileal nutrient digestibility, and energy utilisation in broiler starters fed a sorghum-based diet. Livestock Science 165: 80-86

Abdollahi, M. R., Ravindran, V., Wester T. J., Ravindran G. and Thomas D. V. (2011) Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. Animal Feed Science and Technology 168: 88-99.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G. and Thomas, D. V. (2010) Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize- and sorghum-based diets. Animal Feed Science and Technology. 162: 106-115.

Abdollahi, M. R., Zaefarian, F. and Ravindran, V. (2018a) Feed intake response of broilers: Impact of feed processing. Animal Feed Science and Technology 237: 154-165.

Abdollahi, M. R., Zaefarian, F., Ravindran, V. and Selleb, P. H. (2018b) The interactive influence of dietary nutrient density and feed form on the performance of broiler chickens. Animal Feed Science and Technology 239: 33-43.

Agah, M. J. and Norollahi, H. (2008) Effect of feed form and duration time in growing period on broilers performance. International Journal of Poultry Science 7: 1074-1077.

Allred, J. B., Jensen, L. S. and McGinnis, J. (1957) Factors affecting the response of chicks and poultry to feed pelleting. Poultry Science 36: 517-523.

Amerah, A. M., Ravindran, V. and Lentle, R. G. (2009) Influence of insoluble fibre and whole wheat inclusion on the performance, digestive tract development and ileal microbiota profile of broiler chickens. Poultry Science 50: 366-375.

Amerah, A. M., Ravindran, V., Lentle, R. G. and Thom, D. G. (2007a) Feed particle size: Implications on the digestion and performance of poultry. World’s Poultry Science Journal 63: 439-455.

Amerah, A. M., Ravindran, V., Lentle, R. G. and Thom, D. G. (2007b) Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. Poultry Science 86: 2615-2623.

Amirkolaie, K. A., Jascha, I. L., Johan, A. J. V. and Johan, W. S. (2005) Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (Oreochromis niloticus L.). Aquaculture Research 36:1157-1166.

Annison, G. (1991) Relationship between the levels of soluble non-starch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler chickens. Journal of Agricultural and Food Chemistry 39: 1252-1256.

AOAC International, (2005). Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Washington DC, USA.

Apajalahti, J., Kettunen, A. and Graham, H. (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poultry Science Journal 60: 223-232.

Bartov, I., Bornstein, S. and Lipstein, B. (1974) Effect of calorie to protein ratio on the degree of fatness in broilers fed on practical diets. British Poultry Science 15: 107-117.

Basurco, V., Vieira, **S.** L., Serafini, N. C., Santiago, G. O., Angel, C. R. and Gonzalez-Esquerra, R. (2015) Performance and economic evaluation of feeding programs varying in energy and protein densities for broiler grillers. The Journal of Applied Poultry Research 24: 304-315.

Bayley, H., Summers, J. and Slinger, S. (1968) The effect of steam pelleting feed ingredients on chick performance: Effect on phosphorus availability, metabolizable energy value and carcass composition. Poultry Science 47: 1140-1148.

Behnke, K. C. (1994) Factors affecting pellet quality. Maryland Nutrition Conference, University of Maryland, College Park, MD. pp: 44-54.

Behnke, K. C. (1996) Feed manufacturing technology: current issues and challenges. Animal Feed Science and Technology 62: 49-57.

Behnke, K. C. (2001) Factors influencing pellet quality. Feed Technology 5: 19-22.

Behnke, K. C. and Beyer, R. S. (2002) Effect of feed processing on broiler performance. VIII. International Seminar on Poultry Production and Pathology, Santiago, Chile.

Blakely, R. M., MacGregor, H. I. and Hanel, D. (1963) The effect of type of pelleting on growth and metabolisable energy from Turkey rations. British Poultry Science 4: 261-265.

Bolton, W. (1960) The digestibility of mash and pellets by chicks. The Journal of Agricultural Science 55: 141-142.

Brickett, K. E., Dahiya J. P., Classen, H. L. and Gomis S. (2007) Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. Poultry Science 86: 2172-2181.

Calet, C. (2007) The Relative Value of Pellets versus Mash and Grain in Poultry Nutrition. World's Poultry Science Journal 21: 23-52.

Carré, B. (2004) Causes for variation in digestibility of starch among feedstuffs. World's Poultry Science Journal 60: 76-89.

Cerrate, S., Wang, Z., Coto, C., Yan, F. and Waldroup, P. W. (2009) Effect of pellet diameter in broiler starter diets on subsequent performance. The Journal of Applied Poultry Research 18: 590-597.

Chewning, C. G., Stark, C. R. and Brake, J. (2012) Effects of particle size and feed form on broiler performance. The Journal of Applied Poultry Research 21: 830-837.

Choct, M. (1997) Feed non-starch polysaccharides: chemical structures and nutritional significance. Feed Milling International 191 (June issue) 13-26.

Choct, M. (2009) Managing gut health through nutrition. Poultry Science 50: 9-15.

Choct, M., Hughes, R. J., Wang, J., Bedford, M. R., Morgan, A. J. and Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Poultry Science 37: 609-621.

Classen, H. L. (2016) Diet energy and feed intake in chickens. Animal Feed Science and Technology 233: 13-21.

Coffey, D., Dawson, K., Ferket, P. and Connolly, A. (2016) Review of the feed industry from a historical perspective and implications for its future. Journal of Applied Animal Nutrition 4: 1-11.

Corzo, A., Kidd, M. T., Dozier, W. A., Pharr, G. T. and Koutsos, E. A. (2007) Dietary threonine needs for growth and immunity of broilers raised under different litter conditions. The Journal of Applied Poultry Research 16: 574-582.

Corzo, A., Mejia, L. and Loar, R. E. (2011) Effect of pellet quality on various broiler production parameters. The Journal of Applied Poultry Research 20: 68-74.

Corzo, A., Mejia, L., McDaniel, C. D. and Moritz, J. S. (2012) Interactive effects of feed form and dietary lysine on growth responses of commercial broiler chicks. The Journal of Applied Poultry Research 21: 70-78.

Cowieson, A. J. and Ravindran, V. (2008) Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. Poultry Science 49: 37-44.

Engberg, R. M., Hedemann, M. S. and Jensen, B. B. (2002) The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Poultry Science 43: 569-579.

Farrell, D. J., Cumming, R. B. and Hardaker, J. B. (1973) The effects of dietary energy concentration on growth rate and conversion of energy to weight gain in broiler chickens. British Poultry Science 14: 329-340.

Frikha, M., Safaa, H. M., Jiménez-Moreno, E., Lázaro, R. and Mateos, G. (2009) Influence of energy concentration and feed form of the diet on growth performance and digestive traits of brown egg-laying pullets from 1 to 120 days of age. Animal Feed Science and Technology 153: 292-302.

González-Alvarado, J. M, Jiménez-Moreno, E., Lázaro, R. and Mateos, G. (2007) Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poultry Science 86: 1705-1715.

González-Alvarado, J. M., Jiménez-Moreno, E., González-Sánchez, D., Lázaro, R. and Mateos, G. (2010) Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. Animal Feed Science and Technology 162: 37-46.

Goodarzi Boroojeni, F., Svihus, B., Graf von Reichenbach, H. and Zentek, J. (2016) The effects of hydrothermal processing on feed hygiene, nutrient availability, intestinal microbiota and morphology in poultry-A review. Animal Feed Science and Technology 220: 187-215.

Greenwood, M. W., Cramer, K., Beyer, R., Clark, P. and Behnke, K. (2005) Influence of feed form on estimated digestible lysine needs of male broilers from sixteen to thirty days of age. Journal of Applied Poultry Research 14: 130-135.

Guzman, P., Saldana, B., Mandalawi, H. A., Perez-Bonilla, A., Lazaro, R. and Mateos, G. (2015) Productive performance of brown-egg laying pullets from hatching to 5 weeks of age as affected by fiber inclusion, feed form, and energy concentration of the diet. Poultry Science 94: 249-261.

Hegde, S. N., Rolls, B. A., Turvey, A. and Marie, E. C. (1978) The effects on chicks of dietary fibre from different sources: a growth factor in wheat bran. British Journal of Nutrition 40: 63-69.

Hetland, H., Choct, M. and Svihus, B. (2007) Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poultry Science Journal 60: 415-422.

Hetland, H. and Svihus, B. (2001) Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. Poultry Science 42: 354-361.

Hetland, H., Svihus, B. and Choct, M. (2005) Role of insoluble fiber on gizzard activity in layers. Journal of Applied Poultry Research 14: 38-46.

Hetland, H., Svihus, B. and Krogdahl, A. (2003) Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. Poultry Science 44: 275-282.

Hill, F. W. and Anderson, D. L. (1958) Comparison of metabolisable energy and productive energy determinations with growing chicks. Journal of Nutrition 64: 587-603.

Holsheimer, J. P. and Ruesink, E. W. (1993) Effect on performance, carcass composition, yield, and financial return of dietary energy and lysine levels in starter and finisher diets fed to broilers. Poultry Science 72: 806-815.

Holsheimer, J. P. and Veerkamp, C. H. (1992) Effect of dietary energy, protein, and lysine content on performance and yields of two strains of male broiler chicks. Poultry Science 71: 872-879.

Jackson, S., Summers, J. D. and Leeson, S. (1982) Effect of dietary protein and energy on broiler performance and production costs. Poultry Science 61: 2232-2240.

Jafarnejad, S., Farkhoy, M., Sadegh, M. and Bahonar, A. R. (2011) Effect of crumble-pellet and mash diets with different levels of dietary protein and energy on the performance of broilers at the end of the third week. Veterinary Medicine International Volume 2010, Article ID 328123.

Jahan, M. S., Asaduzzaman, M. and Sarkar, A. K. (2006) Performance of broiler fed on mash, pellet and crumble. International Journal of Poultry Science 5: 265-270.

Jensen, L. S., Merrill, L. H., Reddy, C. and McGinnis, J. (1962) Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. Poultry Science, 41: 1414-1419.

Jensen, L. (2000) Influence of pelleting on the nutritional needs of poultry. Asian-Australasian Journal of Animal Sciences, 13: 35-46.

Jensen, L. S. and McGinnis, J. (1952) A comparison of feeding pelleted and unpelleted diets containing different levels of alfalfa to laying hens. Poultry Science, 31: 307-310.

Jones, R. L. (1986) Nutritional influences on carcass composition in the broiler chicken. Proceedings of the Nutrition Society 45: 27-32.

Jones, R. L. and Wiseman, J. (1985) Effect of nutrition on broiler carcass composition: influence of dietary energy content in the starter and finisher phases. Poultry Science 26: 381-388.

JøRgensen, H., Zhao, X. Q., Knudsen, K. E. B. and Eggum, B. O. (1996) The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. British Journal of Nutrition, 75: 379-395.

Kalmendal, R., Elwinger, K., Holm, L. and Tauson, R. (2011) High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. British Poultry Science 52: 86-96.

Kidd, M. T., Barber, S., Virden, W., Dozier III, W., Chamblee, D. and Wiernusz, C. (2003) Threonine responses of Cobb male finishing broilers in differing environmental conditions. Journal of Applied Poultry Research 12: 115-123.

Kidd, M. T., Corzo, A., Hill, S., Zumwalt, C., Robinson, E. and Dozier III, W. (2005a) Growth and meat yield responses of broilers provided feed subjected to extrusion cooking. Journal of Applied Poultry Research 14: 536-541.

Kidd, M. T., Corzo, A., Hoehler, D., Miller, E. and Dozier III, W. (2005b) Broiler responsiveness (Ross x 708) to diets varying in amino acid density. Poultry Science 84: 1389-1396.

Kilburn, J. and Edwards, H. M., Jr. (2001) The response of broilers to the feeding of mash or pelleted diets containing maize of varying particle sizes. Poultry Science 42: 484-492.

Knudsen, K. E. B. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67: 319-338.

Kuttappan, V. A., Lee, Y. S., Erf, G. F., Meullenet, J. F. C., McKee, S. R. and Owens, C. M. (2012c) Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. Poultry Science 91: 1240-1247.

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W. and Owens, C. M. (2012b) Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poultry Science 91: 2677-2685.

Kuttappan, V. A., Hargis, B. M. and Owens, C. M. (2016) White striping and woody breast myopathies in the modern poultry industry: a review. Poultry Science 95: 2724-2733.

Langhout, D. J., Schutte, J. B., Van Leeuwen, P., Wiebenga, J. and Tamminga, S. (1999) Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Poultry Science 40: 340-347.

Lecznieski, J. L., Ribeiro, A. M. L., de Mello Kessler, A. and Penz Jr, A. M. (2001) Influence of physical form and energy level of the diet on performance and carcass composition of broilers. Archivos Latinoamericanos de Producción Animal 9: 6-11.

Leeson, S., Caston, L. and Summers, J. D. (1996a) Broiler response to energy or energy and protein dilution in the finisher diet. Poultry Science 75: 522-528.

Leeson, S., Caston, L. and Summers, J. D. (1996b) Broiler response to diet energy. Poultry Science 75: 529-535.

Leeson, S., Caston, L., Summers, J. and Lee, K. (1999) Performance of male broilers to 70 days when fed diets of varying nutrient density as mash or pellets. Journal of Applied Poultry Research 8: 452-464.

Lemme, A., Wijtten, P., Van Wichen, J., Petri, A. and Langhout, D. (2006) Responses of male growing broilers to increasing levels of balanced protein offered as coarse mash or pellets of varying quality. Poultry Science 85: 721-730.

Lilly, R. A., Schilling, M. W., Silva, J. L., Martin, J. M. and Corzo, A. (2011) The effects of dietary amino acid density in broiler feed on carcass characteristics and meat quality. The Journal of Applied Poultry Research 20: 56-67.

Liu, H. Y., Ivarsson, E., Jonsson, L., Holm, L., Lundh, T. and Lindberg, J. E. (2011) Growth performance, digestibility, and gut development of broiler chickens on diets with inclusion of chicory (Cichorium intybus L.). Poultry Science 90: 815-823.

Lorenzi, M., Mudalal, M. S., Cavani, C. and Petracci, M. (2014) Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. The Journal of Applied Poultry Research 23:754-758.

Lott, B., Day, E., Deaton, J. and May, J. (1992) The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poultry Science 71: 618-624.

Löwe, R. (2005) Judging pellet stability as part of pellet quality. Feed Technology 9: 15-19.

Mabray, C. J. and Waldroup, P. W. (1981) The influence of dietary energy and amino acid levels on abdominal fat pad development of the broiler chicken. Poultry Science 60: 151-159.

Maiorka, A., Dahlke, F., Santin, E., Kessler, A. M. and Penz, Jr. A. M. (2004) Effect of energy levels of diets formulated on total or digestible amino acid basis on broiler performance. Revista Brasileira de Ciência Avícola 6: 87-91.

Massuquetto, A., Durau, J. F., Schramm, V. G., Netto, M. V. T., Krabbe, E. L. and Maiorka, A. (2018) Influence of feed form and conditioning time on pellet quality, performance and ileal nutrient digestibility in broilers. The Journal of Applied Poultry Research 27: 51-58.

Mateos, G., Jimenez-Moreno, E., Serrano, M. P. and Lazaro, R. P. (2012) Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. The Journal of Applied Poultry Research 21: 156-174.

McDonald, P., Edwards, R., Greenhalgh, J. and Morgan, C. (2011) Animal nutrition (7th edition). Harlow, England; New York: Prentice Hall/Pearson.

McEllhiney, R. R. (1985) Feed manufacturing technology III, American Feed Industry Association, Virginia, pp: 1-11, 529-533.

Moran Jr, E. T. (1982) Starch digestion in fowl. Poultry Science 61: 1257-1267.

Moritz, J. S. and Lilly, K. G. (2010) Production strategies and feeding opportunities for pellets of high quality. Proceedings of the 8th Annual Mid-Atlantic Nutrition Conference, University of Maryland, College Park, MD. pp: 85-90.

Naderinejad, S., Zaefarian, F., Abdollahi, M. R., Hassanabadi, A., Kermanshahi, H. and Ravindran, V. (2016) Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. Animal Feed Science and Technology 215: 92-104.

Nawaz, H., Mushtaq, T. and Yaqoob, M. (2006) Effect of varying levels of energy and protein on live performance and carcass characteristics of broiler chicks. The Journal of Poultry Science 43: 388-393.

Nir, I., Hillel, R., Ptichi, I. and Shefet, G. (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. Poultry Science 74: 771-783.

Nir, I., Twina, Y., Grossman, E. and Nitsan, Z. (1994) Quantitative effects of pelleting on performance, gastrointestinal tract and behaviour of meat-type chickens. Poultry Science 35: 589-602.

Peisker, M. (2006) Feed processing–impacts on nutritive value and hygienic status in broiler feeds. Proceedings of the Australian Poultry Science Symposium 18: 7-16.

Petracci, M., Mudalal, S., Bonfiglio, A. and Cavani, C. (2013) Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poultry Science 92: 1670-1675.

Ravindran, V., Hew, L. I., Ravindran, G. and Bryden, W. L. (2005) Apparent ileal digestibility of amino acids in feed ingredients for broiler chickens. Animal Science 81: 85-97.

Rosa, P. S., Faria Filho, D., Dahlke, F., Vieira, B., Macari, M. and Furlan, R. L. (2007) Effect of energy intake on performance and carcass composition of broiler chickens from two different genetic groups. Revista Brasileira de Ciência Avícola 9: 117-122.

Ross (2014) Ross 308 Broiler: Nutrition Specification, June 2007. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Russo, E., Drigo, M., Longoni, C., Pezzotti, R., Fasoli, P. and Recordati, C. (2015) Evaluation of White Striping prevalence and predisposing factors in broilers at slaughter. Poultry Science 94: 1843-1848.

Saldaña, B., Gewehr, C. E., Guzmán, P., García, J. and Mateos, G. (2016) Influence of feed form and energy concentration of the rearing phase diets on productivity, digestive tract development and body measurements of brown-egg laying hens fed diets varying in energy concentration from 17 to 46 weeks of age. Animal Feed Science and Technology 221: 87-100.

Saldana, B., Guzman, P., Camara, L., Garcia, J. and Mateos, G. (2015a) Feed form and energy concentration of the diet affect growth performance and digestive tract traits of brown-egg laying pullets from hatching to 17 weeks of age. Poultry Science 94: 1879-1893.

Saldana, B., Guzman, P., Safaa, H. M., Harzalli, R. and Mateos, G. (2015b) Influence of the main cereal and feed form of the rearing phase diets on performance, digestive tract, and body traits of brown-egg laying pullets from hatch to 17 weeks of age. Poultry Science 94: 2650-2661.

SAS Institute, (2015). SAS® Qualification Tools User's Guide. Version 9.4. SAS Institute Inc., Cary, NC.

Savory, C. J. (1974) Growth and behaviour of chicks fed on pellets or mash. British Poultry Science 15: 281-286.

Serrano, M., Valencia, D., Méndez, J. and Mateos, G. (2012) Influence of feed form and source of soybean meal of the diet on growth performance of broilers from 1 to 42 days of age. 1. Floor pen study. Poultry Science 91: 2838-2844.

Scott, T. A. (2002) Evaluation of lighting programs, diet density, and short-term use of mash as compared to crumbled starter to reduce incidence of sudden death syndrome in broiler chicks to 35 d of age. Canadian Journal of Animal Science 82: 375-383.

Shen, H., Summers, J. D. and Leeson, S. (1985) Influence of supplemental fat and steam pelleting on the response of broilers to diets varying in energy density. Animal Feed Science and Technology 13: 57-67.

Short, F. J. P., Gorton, J., Wiseman, J. and Boorman, K. N. (1996) Determination of titanium oxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology 59: 215-221.

Singh, Y., Ravindran, V., Wester, T. J., Molan, A. L. and Ravindran, G. (2014) Influence of feeding coarse corn on performance, nutrient utilisation, digestive tract measurements, carcass characteristics, and cecal microflora counts of broilers. Poultry Science 93: 607-616.

Skinner, J. T., Waldroup, A. L. and Waldroup, P. W. (1992) Effects of dietary nutrient density on performance and carcass quality of broilers 42 to 49 days of age. Journal of Applied Poultry Research 1: 367-372.

Svihus, B. (2011) Limitations to wheat starch digestion in growing broiler chickens: a brief review. Animal Production Science 51: 583-589.

Svihus, B. (2014) Function of the digestive system. The Journal of Applied Poultry Research 23: 306-314.

Svihus, B. and Hetland, H. (2001) Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Poultry Science 42: 633-637.

Svihus, B., Kløvstad, K. H., Perez, V., Zimonja, O., Sahlström, S., Schüller, R. B. and Prestløkken, E. (2004) Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarseness by the use of roller mill and hammer mill. Animal Feed Science and Technology 117: 281-293.

Temim, S., Chagneau, A., Guillaumin, S., Michel, J., Peresson, R. and Tesseraud, S. (2000) Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens. Poultry Science 79: 312-317.

Tijare, V. V., Yang, F. L., Kuttappan, V. A., Alvarado, C. Z., Coon, C. N. and Owens, C. M. (2016) Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poultry Science 95: 2167-2173.

Trocino, A., Piccirillo, A., Birolo, M., Radaelli, G., Bertotto, D., Filiou, E., Petracci, M. and Xiccato, G. (2015) Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poultry Science 94: 2996-3004.

Veldkamp, T., Kwakkel, R., Ferket, P. and Verstegen, M. (2005) Growth responses to dietary energy and lysine at high and low ambient temperature in male turkeys. Poultry Science 84: 273-282.

Waldroup, P. W., Mitchell, R. J., Payne, J. R. and Johnson, Z. B. (1976) Characterization of the response of broiler chickens to diets varying in nutrient density content. Poultry Science 55: 130-145.

Walugembe, M., Rothschild, M. F. and Persia, M. E. (2014) Effects of high fiber ingredients on the performance, metabolizable energy and fiber digestibility of broiler and layer chicks. Animal Feed Science and Technology 188: 46-52.

Wiseman, J. and Lewis, C. E. (1998) Influence of dietary energy and nutrient concentration on the growth of body weight and of carcass components of broiler chickens. The Journal of Agricultural Science 131: 361-371.

Zaefarian, F., Abdollahi, M. R. and Ravindran, V. (2016) Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. World's Poultry Science Journal 72: 277-290.

Zimonja, O. and Svihus, B. (2009) Effects of processing of wheat or oats starch on physical pellet quality and nutritional value for broilers. Animal Feed Science and Technology 149: 287-297.